Harmful Algal Blooms, A newly emerging pathogen in water

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Introduction to Cyanobacteria and their Toxins:

Cyanobacteria, or blue-green algae, are ubiquitous in nature and found in nearly all environments. Many species have selective advantages such as the ability to use atmospheric nitrogen for growth or gas vacuoles to control their exposure to light. Cyanobacteria often dominate other algae during the later months of the season, leading to discoloration of the water and surface scums (Figure 1). Cyanobacterial blooms can be associated with taste and odor problems in drinking waters or may lead to allergic responses (i.e. swimmers itch). Cyanobacteria can also produce toxic compounds that inhibit consuming organisms. A look at their historical names- slow death factor, fast death factor and very fast death factor- illustrates their importance. Saxitoxin (STX), one of several toxins produced by cyanobacteria, is a regulated bio-warfare agent and extremely potent (LD50 ~100 μg kbw^{-1}) neurotoxin. A bloom of *Anabaena circinalis* in Australia was responsible for the death of 1600 cattle and sheep. Entire towns where forced to truck in their drinking water to prevent intoxication (Negri et al., 1995).

Figure 1. Bloom of the toxic cyanobacteria *Microcystis* near Venice Beach on Lake Champlain. Blooms such as these have resulted in the death of both pets and wildlife. They have also forced health departments to close the recreational beach to protect tourists from coming in contact with the algae. This results in an economic loss to the surrounding communities.

(*Photograph courtesy of Sylvia Blais, Environment Quebec*)

Toxic peptides produced by *Microcystis* and other species are potent hepatotoxins and have been linked to an increased incidence of hepatic cancer. To prevent human consumption of these toxins through drinking water, the World Health Organization adopted an advisory limit of 1 ug microcystin-LR_{equiv} L^{-1} (Chorus and Bertram, 1999). Cyanobacteria toxins are currently on the EPA's Critical Contaminant List (CCL-2) for regulation and EPA recently completed the necessary toxicological reviews that are a prerequisite for issuing a guideline value. Here we briefly discuss the factors leading to harmful cyanobacterial blooms (HABs), the toxins, current analytical methods, occurrence of toxic blooms and possible management strategies. This review is not meant to be all inclusive or explain all the issues. Its goal is purely to spark discussion amongst the participants.

Factors that Affect the Growth of Cyanobacteria

There are a number of environmental factors that promote the growth of cyanobacterial HABs (Paerl 1996, see Table 1). Generally, either nitrogen (N) or phosphorus (P) is the limiting nutrient in aquatic systems. Enrichment of waters with one or both of these nutrients stimulates algal growth. Phosphorus is often limiting in freshwater systems, including in the Great Lakes, so P inputs from point and non-point sources of agricultural, industrial, and urban origin have contributed to the proliferation of cyanobacterial HABs. These sources can include wastewater treatment facilities, septic tanks, sewer overflow, fertilizer and animal waste runoff from land and atmospheric deposition of aerosolized nutrients. The success of P abatement programs in reducing algal biomass in the late 1980s and early 1990s demonstrates this is an important controlling factor in the Great Lakes region (Makarewicz & Bertram 1991). A less easily controlled nutrient source is the internal loading of P released from anoxic sediments. Iron and other trace metals (Mn, Co, Cu, Zn, etc) are also necessary for cyanobacterial growth (Howarth et al 1988). The concentration and bioavailability of these micro-nutrients may also impact bloom growth and proliferation.

Table 1. Physical–chemical factors potentially controlling blue-green algal blooms (From Paerl 1996)

Factors	Effects and impacts
Physical	
Flushing/altered water residence time	Potential removal mechanism for blooms, if flushing exceeds growth rates of bloom taxa
Large-scale vertical mixing	Counteracts near-surface accumulations of buoyant bloom populations. Forces competition for light and nutrients with more desirable, non-buoyant eukaryotic taxa
Small-scale turbulence (shear)	May disrupt blue-green algal filaments, colonies, aggregates and mutualistic associations with other microflora and micro- and macrofauna
Shading (reduced surface irradiance)	Can alter phytoplankton community composition and can negatively affect blue-green algal surface bloom taxa
Temperature	Generally, temperatures in excess of 20°C accompanied by stratification and high nutrient loading can promote blooms
Chemical	
pH modifications	Can alter phytoplankton community composition; low pH (< 6.0) favours eukaryotes, and high pH (> 8.0) favours cyanobacteria
Nutrient (N and P) inputs	Long-term (months/years) reductions in *both* N and P inputs are frequently effective in reducing blue-green algal bloom potentials; low N:P loading ratios (< 20) often caused by excessive P loading can enhance bloom potential
Salinity	Salinity in excess of a few ‰ (as NaCl) can be an effective barrier to development and persistence of many nuisance species
Trace metals	Under high N and P loading conditions, restricted availability of Fe may control phytoplankton growth; cyanobacteria are able to compete effectively for low levels of Fe; no convincing evidence for other trace metal limitations

Surface water temperatures above 20°C are optimal for cyanobacteria to grow, so blooms are more common in the late summer in the Great Lakes region. *Microcystis* has relatively high light requirements and utilizes gas vacuoles to alter the buoyancy of its cells. This causes the accumulation of cells at the surface of the water and often results in the visible scums that are characteristic of cyanobacterial blooms.

In addition, some cyanobacteria are unpalatable to many zooplankton grazers. This can reduce feeding on the cyanobacteria, or divert the grazing pressure to other, more palatable, phytoplankton species. This lack of zooplankton grazing may be due to a

number of reasons. Cyanobacteria lack sterols and are a low-quality food source for zooplankton (Lampert 1987). Many HAB cyanobacteria are colonial and zooplankton simply are physically unable to ingest the large colonies and filaments. Cyanobacteria also produce peptides that inhibit the digestive enzymes of grazers (Hirada 1994). Simply put, zooplankton usually have more nourishing and better food sources than cyanobacteria on which to feed.

Basic Primer on Toxin Chemistry and Toxic Organisms:

Chemically, cyanobacterial toxins fall into several diverse categories (Table 2). Unfortunately, modern methods of chemical analysis make it very easy to identify new toxins. In 1988, there were 10 reported microcystins. As of 2005, there were more than 85 different microcystin congeners (Figure 2). Microcystins can made by a number of species including *Microcystis*, *Nostoc, Oscillatoria, Anabaena, Planktothrix, Anabaenopsis* and others (Fristachi et al., 2006). In addition to microcystins with 7 amino acids in the cyclic peptide ring, there are closely related nodularin or nodulapeptins with 5 amino acids in the ring. *Microcystis* and other cyanobacteria also make a number of other bioactive peptides such as aeruginopeptin and the anabaenopeptins (Harada et al., 1995). In total, there are probably 300-400 known biologically active peptides produced by cyanobacteria. Many of these peptides are protease inhibitors and interfere with the feeding process of zooplankton. Some like the microcystins are hepatotoxins and protein phosphatase inhibitors. For others, we simply do not know their biological effects at this time. Our analytical techniques in this area have rapidly outpaced our ability to assign biological function.

Table 2: Major toxins produced by cyanobacteria

Name	class	number of forms
Hepatotoxins		
Microcystins	peptide	>85 known
Nodularins	peptide	>6
Neurotoxins		
anatoxin-a	alkaloid	2-4
anatoxin-a(S)	alkaloid	1
Saxitoxin (PSTs)	alkaloid	26
Cytotoxins		
Cylindrospermopsin	alkaloid	4
Dermatoxins		
aplysiatoxins and lyngbyatoxins	polyether alkaloids	80
Neurodegenerative		
β-methyl amino alanine (BMAA)		1

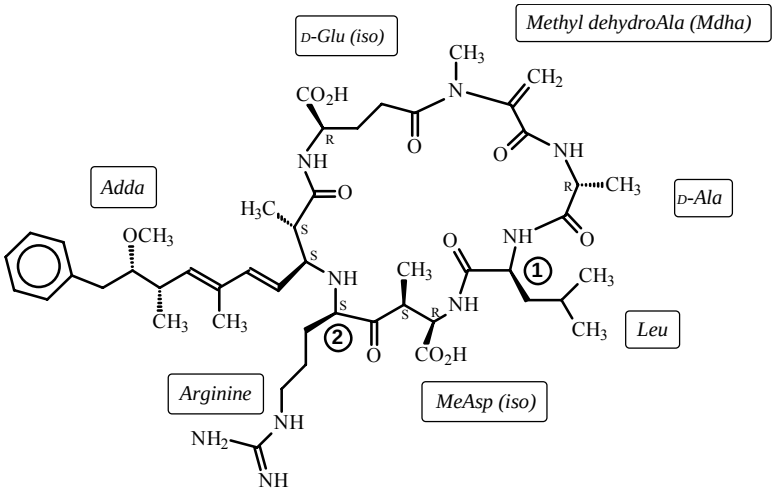

Figure 2. The generic structure of a microcystin. Variations occur primarily at positions 1 and 2. For example, Microcystin-LR contains the amino acids leucine (L) and arginine (R) at positions 1 and 2 respectively. Microcystin-RR has arginine at both positions. Nodularins are similar, with the five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

Separate from the hepatotoxic cyclic peptides are the neurotoxic alkaloids. These consist of anatoxin, anatoxin-a(S), saxitoxin and related analogs (Figure 3). The most important of these compounds from an environmental health aspect is probably anatoxin-a. Anatoxin-a was originally isolated from *Anabaena flos-aquae* but is also produced by a number of other species including *A. planktonica*, *Oscillatoria* species, and *Cylindrospermum*. Homoanatoxin-a, a toxic homologue with a propyl group replacing the acetyl group, was isolated from *Phormidium (Oscillatoria) formosa*. Both are potent nicotinic agonists and act as neuromuscular blocking agents. Anatoxin-a(S) is a naturally occurring organo-phosphate produced by *A. flos-aquae* and *A. lemmermannii*. It acts as an acetyl-cholinesterase inhibitor. Anatoxin-a and -a(S) were likely responsible for the acute toxicity that has resulted in the loss of dogs, birds and cattle in many of the prairie states of the central US and Canada, as well as recent animal poisonings in Ireland and Lake Champlain (Boyer et al. 2004).

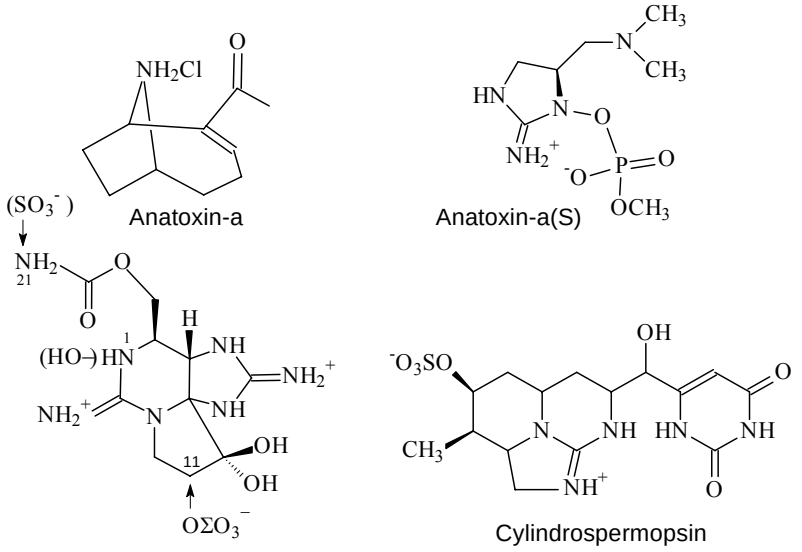

Figure 3. The chemical structure of selected alkaloid neurotoxins produced by cyanobacteria. Anatoxin-a, anatoxin-a(s) and the PST family of toxins, including saxitoxin (STX), neosaxitoxin, and the sulfated gonyautoxins.

Cylindrospermopsin (CYL) has hepatotoxic activity similar to microcystin as well as cytotoxic effects.

Toxic cyanobacteria also contain the Paralytic Shellfish Poisoning Toxins (PSTs), saxitoxin and neosaxitoxin. These toxins are identical to those produced by some "red-tide-forming" marine dinoflagellates. The PSTs are actually a large family of 26 different toxins that are highly variable in their biological activity. Not all of the PST analogs identified in marine sources are present in freshwater cyanobacteria. Saxitoxin is probably the most common, but new toxin analogues have been identified recently. PSTs are produced by selected *Aphanizomenon* species, *Anabaena circinalis, Lyngbya wollei,* and a Brazilian isolate of *Cylindrospermopsis raciborskii*. *Cylindrospermopsis raciborskii* from Hungary or Australia produces a cytotoxic alkaloid called cylindrospermopsin (Figure 3). An outbreak of this organism in the drinking water supply on Palm Island, Queensland Australia, led to a severe outbreak of hepatoenteritis among the inhabitants of the island. Cylindrospermopsin has also been reported from *Umezakia natans* in Japan, and *Aphanizomenon ovalisporum* in Israel (Sivonen and Jones, 1999). Cylindrospermopsin is a major issue for water suppliers in southern Florida and represents a huge management problem for that area (Chapman and Schelske 1997). It was generally assumed toxic cylindrospermopsin-forming species only occurred in tropical and arid environments; however there are reports of *C. raciborskii* occurring in the temperate zones of Europe and the USA (Padisak, 1997, Hamilton et al., 2005). It appears to be gradually moving

northward in its distribution and this species must now be included in any discussion of water problems in the Great Lakes region.

Analytical Methods for the Detection of Cyanobacterial Toxins:

Two points are obvious from the proceeding discussion of toxin chemistry. First, there is an extreme diversity in the number of species producing any given toxin. Not all species are toxic (see below), but a given taxonomic species can vary in the toxins it produces. For example, *Anabaena circinalis* has been shown to be nontoxic, or to produce microcystins, anabaenopeptins, anatoxins and the PSTs. This has important implications for the monitoring of water supplies based on the visual identification of species. The identification of *A. circinalis* in the water column without the chemical confirmation of toxin is meaningless. Second, there is a huge diversity of toxins potentially capable of being produced at any given time. This puts tremendous strain on our analytical capabilities. Basic analytical method for cyanobacterial toxins are divided into two categories. These either group toxins together ("lumper" techniques) or separate the individual toxins ("splitter" techniques) (Table 3). It is important to understand the advantages and limitations between the two approaches, probably more so than any individual technique.

Table 3. Major analytical method for cyanobacterial toxins. The method of choice is shown in bold.

LUMPERS	SPLITTERS
Microcystins	
bioassays	HPLC-PDA
PPIA	LCMS (/MS)
ELISA	
Cylindrospermopsin	
bioassays	HPLC-PDA
ELISA	**LCMS**
Anatoxin-a	
bioassays	**HPLC-FD**
ELISA*	LCMS
PSP toxins (PSTs)	
bioassays	HPLC-FD
ELISA*	LCMS

*newly released techniques

A bioassay, such as the mouse bioassay for toxicity, is a classic "lumper" technique. They give an integrated value for all toxins in the sample, combining the effects of different toxins into a single result. You don't know what toxin(s) are actually in the sample, so assay results are expressed in terms of toxin equivalents. Perhaps the best "lumper" assay for microcystins is the Protein Phosphatase Inhibition Assay or PPIA. Microcystins inhibit PP1a or PP2a as part of their mode of action which can easily be measured in the lab (An and Carmichael, 1994). PPIA gives an extremely good measure of integrated biological activity and commercially available kits for this assay are now available. Another classic "lumper" technique is an ELISA (Enzyme Linked ImmunoSorbant Assays). This technique does not measure biological activity but rather the presence of a structural feature in the molecule. Its use requires caution. Cyanobacterial toxins are too small to trigger the immune response directly and must first be coupled to a carrier molecule to form antibodies. The chemistry used for this coupling process will determine what toxins can be recognized by the final antibody. There are currently four commercially available ELISA kits for microcystins. They were all prepared against microcystin-LR but using different coupling chemistry. As a result, they give very different results depending on what toxin congeners are present in the sample. ELISA assays against the other toxins have had less success. A commercial ELISA assay for saxitoxin has been available for years but shows little to no cross reactivity against the other PSTs found in

cyanobacteria. New ELISAs have been developed for anatoxin-a and cylindrospermopsin. These have yet to be used in the field but are expected to be more successful since there are fewer confounding variations in structure with these two toxins.

To determine what individual toxin variants are present, you must run a chemical analysis or "splitter technique". High pressure liquid chromatography (HPLC) with the appropriate detector is the method of choice. Microcystins can easily be assayed by HPLC using simple UV detection. This is a very non-selective and prone to interferences. A photodiode array detector (PDA) helps to some extent, but most laboratories will want confirmation by looking for the weight of the compound using mass spectroscopy (LCMS). Unfortunately there are only a few standards available for microcystins so it is very difficult to convert these individual results back to the total concentrations in the original sample. Anatoxin-a, cylindrospermopsin and the PSTs such as saxitoxin can all be analyzed using HPLC or LCMS with some tricks. As was the case for microcystins, the availability of high quality standards is a major limitation for all of these techniques.

Anatoxin-a(S) is more problematic. It is very hard to analyze for using HPLC of LCMS because of its structure. The best way to assay for this toxin currently is using the acetyl cholinesterase assay developed for organopesticides. For that reason, most cases of anatoxin-a(S) intoxication are probably reported as organopesticide intoxication and its occurrence is likely under-reported.

Molecular Techniques for the Identification of Potentially Toxic Cyanobacteria

Many phycologists learned the acronym "Anni, Fanni and Mike", referring to the production of anatoxin-a, saxitoxin, and microcystin by *Anabaena flos-aquae, Aphanizomenon flos-aquae*, and *Microcystis aeruginosa.* Unfortunately, the situation is much more complicated. As described above, more than 15 different species of cyanobacteria produce microcystins including the common cyanobacteria genera *Microcystis*, *Anabaena, Oscillatoria (Planktothrix), Nostoc, and Anabaenopsis.* Sorting out who is producing these toxins in a mixed population can be very different, but essential, for application of the proper management techniques. Fortunately molecular techniques such as the polymerase chain reaction (PCR) provide a powerful tool for the study of microcystins. The pathways for toxin biosynthesis and their associated genes have not been identified for the other cyanobacterial toxins at this time, greatly limiting the use of molecular tools for their study.

Microcystins are synthesized differently from most proteins using a group of enzymes called a non-ribosomal peptidyl synthetase (Tillett et al., 2000). These genes are located together in the DNA on what is known as the microcystin (*mcy*) operon (Figure 4). PCR allows us to use specific probes targeted against this operon to identify if the biosynthetic genes for microcystins are present in the sample. Using DNA extracted from the sample, we can tell if the "potential" for microcystin production is present. Using RNA extracted from the sample (rPCR), we can sometimes tell if that potential is being expressed in the form of an enzyme. Potential is not necessarily the same as toxin production, as we have a poor understanding of the regulation mechanisms controlling microcystins formation by the peptide synthetase.

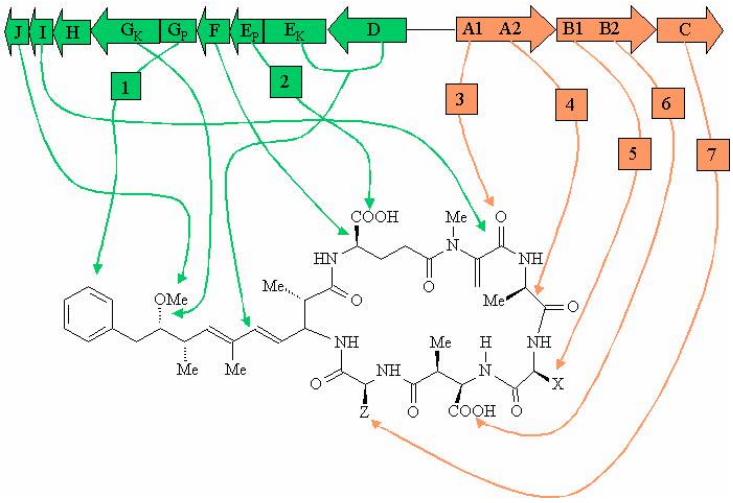

Figure 4. The *mcy* operon for microcystin biosynthesis in *Microcystis aeruginosa.* Genes are designated A-J and are responsible for adding individual pieces of the toxin molecule. *From*: Tillett et al, 2000.

Another important observation is that the *mcy* operon is different in different toxic species. We can exploit those differences to identify which algae in a mixed population of *Microcystis sp.*, *Anabaena sp.*, *Planktothrix sp.* are toxin producers. For example, sequencing selected regions of the *mcy*A operon was used to identify *Planktothrix* species as the major toxins producers in Sandusky Bay (Lake Erie) even in the presence of large numbers of *Microcystis* (Rinta-Kanto and Wilhelm, 2006). This brings up another point readily addressed by molecular techniques. Both toxic and nontoxic strains of these cyanobacteria are commonly found in natural systems at the same time. Since you cannot tell these apart by looking at them, molecular methods have been developed to measure the proportion of the *Microcystis* population capable of microcystin production (Rinta-Kanto et al., 2005). Strains with and without the genetic potential for microcystin production generally co-occur (Kurmayer et al. 2002, Welker et al. 2003), and the relative proportions of each can change with time. This implies that the underlying genetic structure of the population may have a profound effect on the toxicity of a given bloom.

Occurrence of Toxic Blooms in the US

The EPA, in response to the reporting requirements called for in the *Harmful Algal Bloom and Hypoxia Amendments Act* of 2004 (*HABHRCA*) recently completed a scientific assessment of the past occurrence of toxic cyanobacteria in the United States (Fristachi et al, 2006). Cyanobacterial HABs are extremely common and have been documented in 49 of the 50 states. The occurrence of these blooms spans habitats ranging from smaller eutrophic prairie ponds to the larger oligotrophic regions of the Great Lakes. This broad distribution is expected given the diversity in habitats occupied by cyanobacteria and the widespread distribution of potential toxicity in the more common genera. Studies on culture collections and natural blooms in Europe suggest 50% of

Figure 5. Occurrence of toxic cyanobacteria in North America. Carmichael, unpublished, from Fristachi et al, 2006.

all cyanobacterial blooms may contain toxigenic species (Hisbergues et al, 2003). In most cases, environmental factors limit these toxic species to being a minor component of the phytoplankton flora. It is usually only when they bloom and form visible scums that they become problematic and we notice their occurrence.

History of HABs in the Great Lakes

The Laurentian Great Lakes constitute the largest system of fresh, surface water on earth. One-tenth of the US population and nearly 25% of the Canadian population reside in the Great Lakes basin and depend upon its water for consumption, transportation, power, recreation and a host of other uses. In addition to waterborne pathogens, HABs are a significant threat to the water quality of the Great Lakes, its use for recreational activities and drinking water. The Great Lakes region has a history of cyanobacterial blooms, particularly in the shallow (mean depth <8m), productive (mesotrophic-eutrophic) regions such as Saginaw Bay (Lake Huron), the western basin of Lake Erie and the embayments of Lake Ontario (Figure 6). Cultural eutrophication strongly affected these regions during the late 1960s and 1970s, with water-column chlorophyll *a* and total-P concentrations regularly exceeding 20 μg L^{-1} and 1 μM P, respectively. Massive blooms of cyanobacteria (*Anabaena*, *Aphanizomenon* and *Microcystis*) typically dominated the waters during periods of stratification in the summer months (Brittain et al., 2000). Cyanobacterial blooms, chlorophyll *a* and total-P concentrations decreased in frequency and occurrence during the late 1980s and 1990s after aggressive P abatement programs were initiated (Makarewicz & Bertram 1991). Throughout the early 1990s, nuisance algal blooms remained absent from both Saginaw Bay and western Lake Erie only to reappear in 1995 during late summer, this time dominated by *Microcystis* instead of being a mixed cyanobacterial community (Vanderploeg et al. 2001; Brittain et al. 2000). Since 1996, *M. aeruginosa* has again become a dominant component of the summer phytoplankton in Saginaw Bay, often comprising up to 90% of the total biomass. Although *Microcystis* blooms in western Lake Erie are more episodic, expansive blooms have occurred in each of the last four years and microcystin toxin levels have exceeded 20 ug L^{-1} in the Maumee River region (Rinta-Kanto et al, 2005). This reoccurrence of large-scale blooms was a surprise to both researchers and water-resource managers, not only because blooms had not occurred for almost a decade, but also because the cyanobacterial diversity within the blooms had substantially decreased when compared to earlier years. This resurgence of nuisance cyanobacteria has been attributed to the effects of massive populations of dreissenid mussels, changes in the food-web structure, and selective pressure for light and nutrients. Dreissenid mussels (including zebra and quagga mussels) could promote blooms of toxic *Microcystis* through selective rejection of the pseudo-feces during the filtration process

Figure 6. Sites where toxic cyanobacterial blooms have been reported on the Great Lakes. Adapted from Watson et al., 2006

(Vanderploeg et al. 2001) or increasing nutrient recycling and availability in the near shore region (Hecky et al 2003).

Health Effects Associated with Cyanobacterial Toxins:

Bloom-forming algae and the toxins they produce are causative agents for human and animal illness/mortality as well as a litany of environmental-, legal-, and recreational-related problems (Codd 2005). Human health effects associated with cyanobacteria are not new. A recent monogram published by the World Health Organization documents intoxication occurring in the 10^{th} and 12^{th} century (Chorus and Bartram, 1999). Most often, cyanobacteria toxins have been associated with the poisoning of birds and livestock. However, several high profile cases of human intoxication have occurred in recent years. In 1979, the hospitalization of 140 children on Palm Island off Queensland Australian was required due to a massive outbreak of gastroenteritis. Termed the "Palm Island Mystery Disease", its source was traced to the drinking water supplied from a reservoir containing *Cylindrospermopsis raciborskii.* Construction of the Itaparica Dam and reservoir in 1988 in Bahia, Brazil led to another severe gastroenteritis epidemic traced to unknown cyanobacterial toxin(s) in the drinking water. In this event, more than 2,000 cases, and 88 fatalities were reported over a 42 day period. More recently (1996), a cyanobacterial bloom occurred in the water supply for a hemodialysis center in Caruaru, Brazil. Despite extensive pretreatment (sand, carbon, resin and microfiltration), more than 117 of 136 patients (86%) experienced symptoms and 75 patients (55%) died from the incident. This outbreak was termed the "Caruaru Syndrome". In the United States, adverse human affects have included severe gastroenteritis in Pennsylvania where a cyanobacterial-caused disease struck 62 percent of the population served by the Sewickley water utility (Lippy and Erb, 1976). Human illness has also resulted from exposure to *Microcystis* blooms through swimming, boating, and oral ingestion during water sports (Pilotto et al 1997). Nebraska, Massachusetts, Vermont and New York have all closed recreational and swimming beaches in the last few years due to toxic cyanobacterial blooms. Other worries include the widespread distribution of toxic cylindrospermopsin in the Florida surface water supplies and the recent discovery of microcystins in Florida tap water. Chronic exposure to microcystins in untreated drinking water has been related to increased liver damage, possible carcinogenesis and tumor growth promotion. In recognition of the epidemiological data from China, the International Agency for Research on Cancer (IARC) recently changed the classification of microcystin-LR to group 2B; "possibly carcinogenic to Humans" (IARC, 2006).

Fortunately, the effects due to cyanobacteria toxins in the Great Lakes ecosystem have been limited primarily to wildlife and animal fatalities. During 1999 and 2000, large die-offs of waterfowl occurred in Lake Erie and Lake Huron. These deaths were associated with type E or C avian botulism. While the connection with toxic cyanobacteria is tenuous, microcystin-producing cyanobacteria may sensitize the birds to avian botulism (Murphy et al., 2000). The connection between an anatoxin-a producing bloom in Lake Champlain and dog fatalities is more direct. In 1999 and again in 2000, toxic outbreaks of a toxic cyanobacteria resulted in the deaths of several dogs, first from anatoxin-a, and then from microcystin intoxication (Boyer et al, 2004). Microcystins bioaccumulate (but probably not bioconcentrate) in bivalve and fish tissue (Magalhaes et al., 2001), but the significance of this route of exposure is still unknown. The recent resurgence in *Microcystis* blooms in Saginaw Bay and western Lake Erie is of concern due to the widespread use of these waters for recreation, fishing and drinking water.

Management Strategies

Most management strategies for HABs have focused on limiting human exposure to the blooms. Direct control of the harmful algae is usually only applicable in smaller ponds and embayments. Methods that have been used include: (1) adding algicides such as copper sulfate, (2) reducing nutrient inputs, (3) mixing to vertically destratify the water column, (4) reducing retention time by increasing flow rate or flushing, and (5) biological manipulations. Algicides are often used in small areas such as residential ponds over the short term, but due to the potential hazards of these algicides on both aquatic and human health, this approach is generally not recommended. Vertical mixing (such as fountains and bubblers) will have fewer detrimental effects on the system and are again effective in small ponds, but difficult to scale up effectively to larger systems. Algal blooms in dams and impoundments can also be effectively reduced by flushing, but this is not an option for many systems. Biological manipulations, such as adding fish or zooplankton that will feed on cyanobacteria or the addition of lytic bacteria and viruses, have been shown to be successful in specific, localized areas, but the impacts of adding new species to a system can be unpredictable and ultimately more detrimental to the system than the algal blooms themselves. The only option that provides a long-term solution to HAB control without an abundance of other compounding issues is reduction of nutrient inputs. P reduction through detergent bans and increased wastewater treatment was effective in reducing algal growth in the late 1980s and early 1990s in the Great Lakes, and has had similar success in other systems. Even this is not failsafe as some cyanobacterial HAB species make up N deficiencies by using atmospheric N_2 and thus will not be impacted by N reductions to the watershed.

Toxins can also be removed from drinking waters after the blooms have occurred. Chlorination is generally an effective treatment option for removing microcystins, with CT values (product of the disinfectant concentration C in mg L^{-1} and the contact time T in min) that are generally similar to those used for *Guardia* oocysts at lower pH. The effectiveness of chlorine on microcystins greatly decreases at the higher pH (>8.0) often associated with cyanobacterial blooms. Saxitoxin and cylindrospermopsin are also degraded by chlorination, but anatoxin-a appears resistant to chlorine. Other chlorine disinfection processes such as chloramines and chlorine dioxide have shown little promise towards degrading cyanotoxins. Treatment options such ozone, UV or the removal by granulated activated carbon (GAC) or powder activated carbon (PAC) has meet with mixed success. These techniques are negatively affected by the high dissolved organic carbon levels usually associated with cyanobacterial blooms. Wood-based PAC has been used successfully to treat toxic cyanobacterial blooms in the St. Johns Water District in Florida for microcystins and cylindrospermopsin, but no one treatment options appears to work for all toxins (Westrick, 2006).

Conclusions

The occurrence of toxic cyanobacteria in our natural waters is here to stay. Recent changes in both the regulatory environment, our assessments of what is appropriate use for water bodies, and our analytical techniques will likely bring increasing attention to this issue. While monitoring for these toxins is not currently required, the US EPA is currently looking at instituting regulatory guidelines for drinking water. Many other countries have already issued such guidelines for a number of the different toxins and some even have guideline values for recreational contact. Meeting those guideline values will be complicated by the diversity of toxic cyanobacteria present in a water body, by limitations in our current analytical methods, and by a general lack of appropriate treatment strategies once the situation has been diagnosed.

References

An, J and W.W. Carmichael (1994) Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbant assay for the study of microcystins and nodularins. Toxicon. 32:1495-1507.

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

Boyer, G., M. C. Watzin, A. D. Shambaugh, M. F. Satchwell, B. R. Rosen, and T. Mihuc (2004) The occurrence of cyanobacterial toxins in Lake Champlain. In: "Lake Champlain: partnerships and Research in the New Millennium. T. Manley, Ed., p 241-257.

Chapman, A. D., and C. L. Schelske (1997) Recent appearance of *Cylindrospermopsis* (Cyanobacteria) in five hypertrophic Florida Lakes. J. Phycol. 33:191-195.

Codd, C. A., L. R. Morrison, and J. S. Metcalf (2005) Cyanobacterial toxins: risk management for health protection. Toxicol. Appl. Pharamacol. 203:264-267.

Chorus, I., and J. Bartram (1999) Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. World Health Organization, London, NY, 416p.

Fristachi, A., et al., (2006) Occurrence of Cyanobacterial Harmful Algal Blooms Workgroup Report, In H. Kenneth Hudnell (Ed.): Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms Adv Exp Med Biol., in press

Hamilton, P. B., L. M. Ley, S. Dean, and F. R. Pick (2005) The occurrence of the cyanobacterium *Cylindrospermopsis raciborskii* in Constance lake: an exotic cyanoprokaryote new to Canada. Phycologia. 44:17-25.

Harada, K., K. Fujiki, T. Shimada, M. Suzuki, H. Sano, K. Adachi, et al. (1995) Two cyclic peptides, anabaenopeptins, a third group of bioactive compounds from the cyanobacterium *Anabaena flos-aquae* NRC 525-17. Tetrahedron Lett. 36:1511-1514.

Harada K (2004) Production of secondary metabolites by freshwater cyanobacteria. Chemical & Pharmaceutical Bulletin. 52 889–99.

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.

Hisbergues, M., G. Christiansen, L. Rouhiainen, K. Sivonen, and T. Borner (2003) PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. Arch Microbiol. 180:402-410.

Hecky, R.E., R.E.H. Smith, D.R. Barton, S.J. Guildford, W.D. Taylor, M.N. Charlton and T. Howell. (2003) The nearshore phosphorus shunt: a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. 61:1285-1293.

Howarth, R.W., R. Marino and J.J. Cole (1988) Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. 33:688-701.

IARC (Intern. Agency Research Cancer) (2006) Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. The Lancet Oncology. 7:628-629.

Kurmayer, R., E. Dittmann, J. Fastner and I. Chorus (2002) Diversity of microcystin genes within a population of the toxic cyanobacterium *Microcystis* spp. in Lake Wannsee (Berlin, Germany). Microbial Ecology 43:107-118.

Lampert, W. (1987) Laboratory studies on zooplankton-cyanobacteria interactions. NZ J. Mar. Freshwater Res. 21:483-490.

Lippy E.C. and J. Erb (1976) "Gastrointestinal illness at Sewickley, Pa." J Am Water Works Assoc 68: 606-610.

Makarewicz, J. C. and P. Bertram (1991) Evidence for the restoration of the Lake Erie ecosystem. Bioscience 41:216-223.

Magalhaes. V.F., R.M. Soares and S.M. Azevado (2001) Microcystin contamination in fish from the Jacarepagua Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon. 39:1077-1085.

Negri, A. P., G. J. Jones, and M. Hindmarsh (1995) Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium Anabaena circinalis. Toxicon. 33:1321-1329.

Padisak, J. (1997) *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. Arch Hydrobiol., Suppl. 107:563-593.

Paerl. 1996. A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. Phycologia 35 (suppl.):25-35.

Pilotto, L.S., Douglas, R.M. Burch, M.D., Cameron, S., Beers, M., Rouch, G.R., Robinson, P., Kirk, M., Cowie, C.T., Hardiman, S., Moore, C., and R.G. Attewell (1997) Health effects of recreational exposure to cyanobacteria (blue-green algae) during recreational water-related activities. Aust. N. Zealand J. Public Health. 21: 562-566.

Sivonen, K., and G. Jones (1999) Cyanobacterial Toxins. In: "Toxic Cyanobacteria in Water" I. Chorus, and J. Bartram, Eds., World Health Organization, London, pp. 41-112.

Tillett, D., E. Dittmann, M. Erhard, H. von Dohren, T. Borner, and B. A. Neilan (2000) Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC 7806: an integrated peptide-polyketide synthetase system. Chemistry and Biology. 7:753-764.

Rinta-Kanto, J. M., and S. W. Wilhelm (2006) Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. Appl Environ. Microbiol. 72:5083-5085.

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

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

Welker, M., H. von Dohren, H. Tauscher, C.E. Steinberg and M. Erhard (2003) Toxic *Microcystis* in shallow Lake Muggelsee (Germany) - dynamics, distribution, diversity. Arch. Hydrobiol. 157:227-248.

Watson, S. B., G. L. Boyer, and E. Hinchey (2006) Naturally produced noxious chemicals and toxins in the Great Lakes. State of the Lake Ecosystem (SOLEC) 2006. Milwaukee WI, November 1-3, 2006.

Westrick, J.A. 2006, Cyanobacterial toxin removal in drinking water treatment processes and recreational waters In: Hudnell (Ed.): Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms Adv Exp Med Biol., in press