TOXIC CYANOBACTERIA (BLUE-GREEN ALGAE): AN EMERGING CONCERN

Robert C. Hoehn, Ph.D.
Emeritus Professor
The Charles E. Via, Jr. Department of Civil and Environmental Engineering
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

Bruce W. Long, P.E.
Vice President
Director, Water Treatment Technology
Black & Veatch
Kansas City, Missouri 64114

INTRODUCTION

Cyanobacteria belong to a group of prokaryotic organisms identified by a variety of names, including cyanobacteria, blue-greens, blue-green algae, myxophyceans, cyanophytes, cyanophyceans, and cyanoprokaryotes. These primitive and highly adaptable organisms are most often referred to as either blue-green algae or cyanobacteria. Both terms are equally acceptable and can be used interchangeably. This group of organisms is unique among the bacteria in that they contain chlorophyll, and, therefore, are most likely the progenitors of true algae.

Historically, water treatment personnel in the USA have regarded cyanobacteria only as nuisance organisms associated with earthy and musty tastes and odors and unsightly conditions in the water supply. The only drinking water regulation that currently applies to them in the USA is an unenforceable secondary maximum contaminant level (SMCL) for the threshold odor number (TON) of 3 (*Federal Register*, 1979). In other countries, however, toxin production by some cyanobacteria has long been recognized and is regarded as a serious issue.

Carmichael (1992) attributed the apparent lack of concern in the USA to the fact that no link has ever been made between cyanobacterial toxins (cyanotoxins) in water supplies and human deaths or illnesses. Drinking water suppliers, regulators, and the public are generally unaware of potential problems that toxic cyanobacteria can cause, and neither the current nor pending regulations even mention them. The situation is changing, however. In 1998, the United States Environmental Protection Agency (USEPA) included freshwater cyanobacteria and their toxins on the first Candidate Contaminant List (CCL) (*Federal Register*, 1998). This list includes organisms and chemicals selected on the basis of their potential public health significance and general unavailability of occurrence data and other relevant information. Cyanobacteria and their toxins were included in the CCL for two reasons, namely that (1) they are not necessarily associated with fecal contamination, and,

therefore, will not be adequately controlled by provisions of either the Surface Water Treatment Rule or the Enhanced Surface Water Treatment Rule, and (2) they may not be adequately removed from drinking water by conventional water treatment techniques. Listing these organisms in the CCL, therefore, will focus attention on them and "make them a priority for research to determine what triggers toxic algae growth in source water and the effectiveness of water treatment practices" (*Federal Register,* 1998).

Critical information regarding cyanobacteria and their toxins (e.g. occurrence, analytical methods, and health effects) must be available before the USEPA can begin to consider regulations for them. This information can be collected under the provisions of the 1999 Unregulated Contaminants Monitoring Regulation (UCMR) discussed in Section 1412 (1)(b)[b] of the 1996 Safe Drinking Water Act Amendments.

In May, 2001, a panel of experts was convened by USEPA in Cincinnati to assist the agency in developing a target list of cyanotoxins and assigning priorities for future action. The Office of Drinking Water and Ground Water is currently reviewing the panel's recommendations. Once the list is finalized, the prescreen portion of the UCMR will be used to develop occurrence data and evaluate analytical methods for the cyanobacteria and their toxins. Vulnerable water systems will be selected for monitoring, and analytical methods for toxin analyses will be developed and evaluated. In 2001, an entire session at the AWWA Water Quality Technology Conference (AWWA, 2001) was dedicated to papers by international experts in the area of cyanobacteria and their toxins.

This paper discusses the following: (1) the biology of cyanobacteria, their association to "hazardous algal blooms (HABs)" in water supplies, the toxins that some species produce, and the methods for their detection and quantification, (2) available cyanobacterial bloom-control methods, (3) effectiveness of water treatment processes for cyanotoxin removal, (4) international guidelines for cyanotoxins in drinking water and the priorities the USEPA panel assigned to these toxins, and (5) the results of an recently completed assessment of cyanotoxins in North American drinking water supplies. Additional information regarding these and other cyanobacteria topics are available in a 1999 World Health Organization publication (Chorus and Bartram, 1999), and two American Water Works Association Research Foundation project reports (Yoo et al., 1995; and Carmichael, 2001).

CYANOBACTERIA: ECOLOGY AND TOXIN PRODUCTION

Ecology

The dominant algal populations in temperate-zone surface waters change with the seasons. Diatoms and small flagellated algae typical dominate in winter and early spring followed by green algae in the late spring and early summer. In late summer and early fall, the dinoflagellates (pyrrophytes), large yellow-green algae (chrysophytes), and desmids are dominant. If the impoundments are eutrophic, the blue-green algae begin to emerge in the late summer when the water temperature increases and dominate the algal community until

the water temperature decreases in the winter. Seasonal population differences are small in tropical lakes, and blue-green often are the dominant type most of the year.

Cyanobacteria grow best in nonturbulent, warm rivers, lakes and reservoirs. Blooms usually occur during the warmest months of the year, especially when the water contains an over-abundance of nitrogen (N) and phosphorus (P). Excessive P most often provides the stimulus for cyanobacterial blooms, especially if the total N to total P concentration ratio is less than 10. Cyanobacteria with special structures called heterocysts can fix atmospheric nitrogen, and, in these species, nitrogen is seldom limiting. Carmichael (1994) described a bloom as an explosion of growth that occurs when light, temperature, and nutrients favor one species, resulting in cell densities of several million per liter.

Another physiological feature that gives several cyanobacteria species a competitive edge in aquatic ecosystems is their ability to migrate vertically in the water column. Special gas-filled structures called "vesicles" are found within vacuoles inside the cells. Filling and emptying of these structures, along with the production and utilization of a high-molecular-weight carbohydrate in the vacuoles, account for cell buoyancy. The accumulation of massive amounts of cell material on the surface is referred to as a "scum."

Dr. Justin Brookes (Australian Centre for Water Research, South Australia, personal communication, 2001) described the vertical migration process as follows:

> *Vertical migration is often viewed as a mechanism to scavenge for the vertically separated resources, light and nutrients. The mechanism for buoyancy regulation and vertical migration is mostly due to the accumulation (and loss) of carbohydrate via photosynthesis (and respiration). The carbohydrate is actually the intermediates of photosynthesis (sugars) and has a density of about 1600 kg/m^3. Consequently this acts as ballast and if sufficient is accumulated this may overcome the buoyancy provided by gas vesicles and the cell will tend to sink. Because photosynthesis is light dependent the accumulation of carbohydrate also changes in response to the light intensity.*
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> *Cells in the early morning have low carbohydrate as they have been in the dark respiring carbohydrate - consequently they are buoyant. As solar insolation increases, the cells phostosynthesize, accumulate carbohydrate and tend to sink. As they migrate vertically they see less light and so the rate of carbohydrate accumulation decreases until the compensation irradiance where photosynthesis and respiration are equal. At lower light intensities respiration uses the carbohydrate and buoyancy is restored. In reality [vertical migration] is more complex than this and the short-term response to light is nested within a longer-term response to nutrients and light which affects gas vesicle volume etc.*

Toxin Production

Toxic cyanobacteria are only one of several algae that cause what are referred to as "harmful algal blooms" (HABs), and they have been found on every continent except Antarctica. Other toxic algae include certain dinoflagellates (pyrrophytes) that cause the red tide, resulting in fish deaths and rendering inedible a variety of fish and shellfish. Only about 2% of the many dinoflagellate species are toxic. Another toxic dinoflagellate, *Pfiesteria piscicida* (pronounced fee-STEER-ee-uh pis-kuh-SEED-uh), has wreaked havoc in estuaries from Delaware to North Carolina. The species name, *piscicida*, means "fish killer," and its toxins cause fish lesions and death of aquatic organisms living in brackish and saline waters. *Pfiesteria piscicida* was first discovered in 1988 by N.C. State researchers. Unlike most of the dinoflagellate toxins, *Pfiesteria* toxins are extracellular and are used to stun fish. More information about these organisms can be found on the web at the following web sites:

www.nal.usda.gov/wquic/pfiest.html and www.epa.gov/owow/estuaries/pfiesteria

The term HAB has been used to describe toxic cyanobacterial blooms as well as red tide and *Pfiesteria* blooms. In 2000, public attention was focused on toxic cyanobacteria in Florida when blooms of *Cylindrospermum raciborskii* occurred in several lakes. In 1998, prior to the cyanobacterial blooms in water supplies, Florida established an HAB Task Force to address concerns over red tide and *Pfiesteria* blooms in costal waters, and this group became the major source of public information related to toxic cyanobacteria when the blooms occurred. A toll-free hotline for reporting possible illnesses caused by these organisms and a web site were established to provide operation and address public concerns. Numerous web sites on various aspects of cyanobacterial toxins are available.

Toxic and nontoxic cyanobacteria are indistinguishable by microscopic examination, and both kinds may be in the same water bloom. Environmental conditions that foster toxin production are not well understood, and sophisticated tests are required for determining whether or not a bloom contains toxic species, and (Mur et al., 1999). The most common freshwater cyanotoxic genera are *Anabaena, Aphanizomenon,* and *Microcystis* bit others include *Oscillatoria, Lyngbya, Planktothrix, Phormidium*, and *Anabaenopsis*. *Cylindrospermum raciborskii*, which produces a hepatotoxic alkaloid, was the organism that was found in Florida lakes in 2000.

HISTORICAL OVERVIEW OF CYANOTOXIN EPISODES

Francis (1878) published the first report of deaths caused by toxic cyanobacteria, but animals, not humans, were involved. Numerous cattle, sheep, and horses died after they drank water from Lake Alexandrina near Adelaide, South Australia as it was experiencing a massive blue-green algal bloom. The agent was identified as *Nodularia spumigena*, which was later confirmed as the toxic agent by feeding it to a calf that subsequently died. Since then, numerous reports of animal and bird deaths have been published, but most events have been caused by toxins other than nodularin (Ressom et al., 1994; Kuiper-Goodman et al., 1999; WHO, 1999).

Kuiper-Goodman et al. (1999) and Carmichael (1992) described several instances of cyanotoxin-related illnesses in humans dating back to 1931. Some of the events occurred after massive cyanobacterial blooms were treated with copper sulfate, which caused the cells to lyse and release the intracellular toxins. While toxins are released at all stages of their life, senescent cyanobacteria and those killed by copper sulfate liberate excessive quantities of toxins that can be difficult to remove by drinking water treatment.

Poisonings are classified as either acute or chronic, but most human poisonings are chronic**.** The effects of acute poisonings occur very soon after exposure and include severe gastroenteritis, nausea, vomiting, diarrhea, and fever but rarely death (Carmichael, 1992). Numerous deaths did occur in Caruaru, Brazil, however, when 136 dialysis patients were exposed to microcystins in water used for the dialysis. Of these, 100 experienced liver failure, and 50 died (Kuiper-Goodman et al., 1999; Jochimsen et al., 1998). Potential chronic effects, which develop over a long period of exposure, include chronic liver damage and even carcinoma (Kuiper-Goodman et al., 1999). These are of greater concern because treated drinking water supplies would contain low levels of the toxins, not levels high enough to cause death. The possibility of liver cancer following exposure to cyanobacterial toxins is of special concern. Falconer (1983) monitored liver enzyme levels in a group of Australian men, women and children whose water supply was a reservoir that was plagued annually by cyanobacterial blooms. Following a massive *Microcystis spp.* bloom, elevated liver enzyme levels, which indicate liver damage, were found in many of the test subjects who drank treated water taken from the impacted reservoir. Yu (1989) studied the incidence of liver cancer in China and reported that the cancer rate was significantly higher among people who drank water from ditches containing massive blooms of cyanobacteria than in people in the same area who drank groundwater.

Direct contact with cyanobacterial toxins in recreational lakes and reservoirs can also adversely affect humans by causing skin irritation and increasing the risk of gastrointestinal symptoms if the water is swallowed. Allergic responses to direct toxin exposure also can occur. Such incidents have been documented in Canada, the United Kingdom, and Australia (Kuiper-Goodman et al., 1999). Carmichael (2001) described numerous episodes of similar human poisonings brought about by direct contact with toxic cyanobacterial blooms in surface waters.

CYANOTOXINS: CLASSIFICATION, EFFECTS, AND ANALYTICAL METHODS

Classification and Effects

Cyanotoxins can be classified according to the animal organs they affect and their according to their chemical structure. Neurotoxins affect the nervous system, hepatotoxins attack the liver, and dermatoxins irritate skin and mucous membranes. The three most-common cyanotoxins are described chemically as cyclic peptides, alkaloids, and lipopolysaccharides (LPS) (Mur et al., 1999).

Cyclic peptides. Most cyanobacterial poisonings are caused by a group of small-molecular-weight cyclic peptides known as microcystins and nodularins (Carmichael, 1997). These compounds are produced by certain species of *Nostoc, Microcystis*, *Anabaenopsis, Anabaena*, and *Oscillatoria*. Some *Anabaena* and *Oscillatoria* species also produce odor-causing compounds, but no statistical correlation has been found between toxin concentrations of the odor compounds in water supplies (Hrudey et al., 1993; Carmichael, 2001). Carmichael (2001), however, found a high percentage of raw water supplies containing cyanotoxins during blue-green algal blooms also were experiencing taste-and-odor problems at the time the waters were tested for toxins.

Microcystins, which is the most common class of toxins worldwide, was first isolated from *Microcystis aeruginosa,* the organism from which their name is derived (Carmichael, 1988). Other *Microcystis* species that produce these toxins are *M. viridis* and *M. wesenbergii*, but *M. aeruginosa* is the species most often identified with freshwater cyanobacterial poisonings (Carmichael, 2001). Approximately 65 structural variants of microcystin have been described (Mur et al. 1999). The most common is microcystin-LR, ("L" means leucine and "R" means arginine). Certain *Anabaena* and *Oscillatoria* species also produce microcystins. Carmichael (1992) described the symptoms of acute hepatotoxin poisonings in animals as whitening of the mucous membranes, vomiting, cold extremities, and diarrhea. Death occurs by hemorrhage within liver tissue. Acute poisonings are not expected in human populations except in rare cases where massive amounts of cellular material would be ingested. Chronic poisonings in humans are more likely and are indicated by gastrointestinal upsets, diarrhea, vomiting, and, in the worst case, liver cancer.

Nodularins, the other cyclic peptide toxin group, are produced by only one cyanobacterial species, *Nodularia spumigena*, which is most found predominantly in the Baltic Sea and brackish estuaries and in costal lakes in New Zealand and Australia (Mur et al. 1999). The nodularins are generally unimportant in fresh waters.

Alkaloid toxins. Both neurotoxic and cytotoxic alkaloids have been identified. The major neurotoxic alkaloids are the anatoxins and saxitoxins, and the major cytotoxic alkaloid is cylindrospermopsin. Only acute poisonings involving these toxins have been studied, and the effects of chronic exposures are not known.

The anatoxins include anatoxin-a (the "fast-death factor"), anatoxin-a(S), and homoanatoxin-a. Each affects nerves and interferes with the smooth transition of stimuli to the muscles. Anatoxin-a has been isolated from species of Anabaena, *Oscillatoria*, and *Aphanizomenon* species. Anatoxin-a(S) (S means "salivation factor") occurs primarily in *Anabaena* species, including *A. flos-aquae*, *A. spiroides*, and *A. circinalis* (Carmichael, 1992; 2001). Animals that ingest large quantities of cyanobacteria that produce these toxins die in a matter of seconds. Death is caused by paralysis of the respiratory muscles but is often preceded by leaping, staggering, muscle twitching, gasping, and convulsions. Anatoxin-a(S) causes profuse salivation in addition to the other manifestations of anatoxin-a poisoning. Large animals can be poisoned by drinking only a few liters of neurotoxin-containing water while small birds and mammals can be killed if they ingest only a few

milliliters (Carmichael and Gorham, 1977; Carmichael et al., 1977; Carmichael and Biggs, 1978).

The neurotoxin saxitoxins are also known as Paralytic Shellfish Poisonings (PSPs) and include the both the saxitoxins and neosaxitoxin. Sixteen saxitoxins have been identified so far. (Carmichael, 2001). Dinoflagellates such as those that cause the red tide phenomenon and *Pfiesteria piscicida* are the best known producers of these toxins. As mentioned earlier, they pose no threat to human health through public water supplies. Some freshwater cyanobacteria - including *Aphanizomenon flos-aquae*, *Anabaena circinalis*, and *Lyngbya wollei* - also produce neurotoxic saxitoxins (Mur et al. 1999). Certain strains of *Aphanizomenon flos-aquae*, which thus far have been found only in New Hampshire, produce both saxitoxins and neosaxitoxin (Carmichael, 2001). Mur et al. (1999) mentioned that freshwater mats of PSP-producing *Lyngbya wollei* have been found in southern and south-central reservoirs and lakes in the USA. Saxitoxins and related neurotoxins produced by massive blooms of *Anabaena* spp. were responsible for the death of 1600 cattle and sheep along the Murray River in Australia in 1990 (Humpage et al. 1993).

The cytotoxic alkaloid cylindrospermopsin affects mainly the liver and is found most often in blooms occurring in tropical, subtropical, and arid regions. It was identified after-the-fact as the cause a 1979 poisoning, which at the time was referred to as 'Palm Island mystery disease" because the cause of the problem was not readily identified in either foods or fecal samples from the affected populace. In all, 140 children and 10 adults were affected, and while none died, extensive therapy was required for most of them. An epidemiological study revealed that the drinking-water source used by all the victims was Solomon Dam, and individuals on other water supplies were unaffected. Prior to the outbreak, a bloom of *Cylindrospermum raciborskii* had been discovered in the dam reservoir and treated with copper sulfate. The algicide caused the cells to rupture releasing large quantities of cylindrospermopsin into the water (Bourke et al., 1983; Kuiper-Goodman et al., 2001). The affected children suffered symptoms of toxin exposure toxin that included "malaise, anorexia, vomiting, headache, painful liver enlargement, initial constipation followed by bloody diarrhoea and varying levels of severity of dehydration" (Kuiper-Goodman et al., 2001).

Since the Palm Island episode, *Cylindrospermum raciborskii* has been isolated from other lakes in Australia and in Hungary. Cylindrospermopsin has been isolated from other cyanobacteria, including *Umezakia nata* in Japan and Aphanizomenon *ovalisporum* in Israel. Increasing numbers of occurrences of *C. raciborskii* blooms have been reported in Europe and Asia (Mur et al. 1999).

Lipopolysaccharides (LPS). These toxins are in a class of toxins called "endotoxins" and are often referred to as "irritant toxins" (Sivonen & Jones, 1999). They are produced by cyanobacteria whose cell-wall constituents are similar to but less potent than the toxins found in Gram negative bacteria such as *Salmonella*. They were first isolated from cyanobacteria by Weise *et al.* (1970). The toxins can cause irritant and allergenic responses in humans who come in contact with them. The main effects are the result of direct contact

rather than ingestion, but the health risks are not well understood at this time (Sivonen & Jones, 1999). They are of special interest in Australia but have not received much attention in the USA.

Analytical Methods

Methods for identification and screening are available (Yoo et al. 1995), but the identification methods (e.g. liquid chromatography followed by mass spectroscopy) require sophisticated instrumentation and analytical skills most likely unavailable only in commercial and research laboratories. Screening tests, which are simpler and most practical for consideration by water utilities, include: (1) enzyme-linked immunosorbent assays (ELISA) for which commercial kits are commercially available and which are the most ubiquitous (Carmichael 2001), and (2) protein phosphatase inhibition (PPI) assays for which commercial kits are potentially forthcoming. The ELISA kits can be used for quantification.

Carmichael (2001) used a "polyclonal MYCYST-LR antibody test kit, which is available from several commercial suppliers. Information from one supplier (EnviroLogix Inc.; Portland, Maine) describes the test as being one in which "microcystin toxin competes with enzyme (horseradish peroxidase)-labeled microcystin for a limited number of antibody binding sites immobilized to the inside surface of the test wells." The microcystin concentration is related to the color intensity that develops on strips to which sample water and reagents have been added. The color is compared to a chart, and lighter colors indicate higher concentrations. The limit of detection (LOD) for four microcystins varies from 0.15 μg/L to 0.44 μg/L.

The ELISA kits, which at present are mainly for microcystins, are available from EnviroLogix, Inc. in Portland, Maine, and CyanoLab in Palatka, Florida. The contact at EnviroLogix at this writing is Mr. John Chamberlain at (207) 797-0300, extension 428, and the CyanoLab contact is Mr. John Burns, (366) 3280-9646. Each company has a web site.

Mouse bioassays are also used for screening cyanotoxins in cells and water, but they are difficult, expensive, and impractical for consideration at all but the largest research laboratories. Detailed discussions of all the analytical and screening methods for cyanotoxins are available elsewhere (Yoo et al., 1995; WHO, 1999).

USEPA Advisory Panel Toxin Prioritization for Future

The advisory panel that USEPA convened in 2001 to advise the agency on matters related to cyanobacteria prioritized the cyanotoxins for future studies. The highest priorities were given to microcystins, cylindrospermopsin, and anatoxin-a. The saxitoxins and anatoxin-a(S) were assigned medium–to-high priority, while nodularin, lyngbyatoxin and a host of other less-commonly found toxins were listed as "needing additional study." The LPS toxins were not assigned any priority because the panel felt they did not pose problems in US drinking water supplies.

CYANOTOXIN HAZARDS, RISK ASSESSMENTS, AND GUIDELINES

Hazards

While direct evidence is lacking, some past public-health episodes may have been related to cyanobacteria in drinking water supplies. Tisdale (1931) published the earliest report of suspected cyanobacterial poisonings in the USA. Several thousand Charleston, West Virginia, residents who drank city water experienced acute gastroenteritis following massive cyanobacteria growths in the Kanawa River from which their water supply was derived. While the illnesses could not be directly linked to the cyanobacteria, epidemiological studies failed to find any enteric pathogens. Public health officials noted that the outbreak followed a heavy growth of algae associated with taste-and-odor problems in the water supply. In 1976, another outbreak of gastroenteritis occurred among 62% of the population over a 6-day period in Sewickley, Pennsylvania (Lippy and Erb, 1976). Similar episodes have been reported in Canada, Australia, the United Kingdom, and South Africa (Carmichael, 2001).

Risk assessments

Acute lethal injury caused by cyanotoxins in water is unlikely in the United States, and chronic exposure is potentially of greater concern. Carmichael (2001) pointed out (1) that risk assessments are possible only when the expected frequencies of undesirable effects brought about by exposure to a toxicant can be estimated and (2) that the term "hazard" is more appropriate when either quantification data or rates of toxic effects derived during actual studies are lacking. He further noted that most toxin exposures occur primarily in one of two ways: (1) direct contact with water containing the toxins and cyanobacteria or direct ingestion of water by swimmers and water skiers during recreational activities and (2) eating dietary supplements made with cyanobacteria. Some people are exposed during showering by inhalation of water droplets containing toxins, but Carmichael considered this exposure route to be minor.

Tumor promotion by exposure to cyanobacterial toxins poses an added risk and must be factored into risk calculations along with data from animal studies and toxicological and chemical information. Few data are available regarding human exposure to cyanobacterial toxics through drinking water.

Studies leading to the establishment of a drinking water standard for microcystins in terms of either a no-adverse-effect level (NOAEL) or maximum acceptable concentration (MAC) are being conducted by public health authorities in Australia, Canada, and Great Britain. Canadian authorities have proposed a MAC of 0.5 μg/L for m-LR. In the absence of "potency equivalency values" for other microcystins, Canadian authorities have proposed 1 μg/L for total microcystins. Australian authorities, using toxicological results from pig-feeding studies (Falconer 1994) and incorporating a safety factor for tumor promotion, established a NOAEL for microcystins and nodularins at 1.0 μg/L (Carmichael, 2001)

Falconer et al. (1999) described the method that is used in calculations of an exposure guideline for m-LR based on the tolerable daily intake (TDI), adult body weight, and the percentage of the TDI that occurs through a daily drinking water intake of 2 L/d. Based on available data, WHO published a provisional value of 1.0 μg/L (WHO, 1998; 1999). The authors noted that the guideline applied only to m-LR because data on which to base a guideline for all the cyanotoxins were unavailable.

Guidelines

At present, the USA has established no guidelines for any of the cyanotoxins. Germany and New Zealand have a microcystin guideline of 1.0 μg/L, which is the same as the WHO guideline (WHO, 1998, 1999). Canada's total microcystin guideline is 1.5 μg/L, while Australia's is1.3 μg/L in terms of m-LR toxicity equivalents. The United Kingdom's position on guidelines is that the individual water utilities are bound by law to protect the consumers, so no need exists for the government to become involved. Australia has suggested 3 μg/L as potential guidelines for both anatoxin-a and the saxitoxins and is considering a cylindrospermopsin guideline in the range of 1-15 μg/L.

MANAGING POTENTIAL RISKS WITH BLOOM CONTROL AND DRINKING WATER TREATMENT

Human risks of cyanotoxin exposure through drinking water can be minimized by prevention of cyanobacterial blooms in waters supplies and removing the toxins during drinking water treatment.

Bloom control

The most effective weapon against the occurrence of cyanobacterial toxins in water supplies obviously is bloom prevention. Because inorganic nutrients (nitrogen and phosphorus) are usually responsible for blooms, good watershed management to prevent their influx to raw water supplies (*Federal Register, 1998).* However, eutrophic lakes and reservoirs that have been impacted by years of poor watershed management and excessive nutrient loadings improve slowly after best management practices are in place. Lake sediments usually are laden with phosphorus, and in deep lakes, the phosphorus can be released when the bottom becomes anaerobic during the summer. For this reason, much of the research into cyanobacterial toxin control has focused not on watershed management but rather on the effectiveness of various in-plant treatment processes for removing the toxins.

Numerous cyanobacterial bloom-control measures have been summarized by Chorus and Luuc (1999) and by Yoo et al. (1995). As was mentioned earlier, toxic and nontoxic cyanobacterial species are indistinguishable by microscopic examination, so control of all blooms should be everyone's goal. The potential for toxin production by the same organisms that cause taste-and-odor problems has raised the issue of cyanobacterial bloom control to a new level of importance.

Many drinking water utilities apply copper sulfate and copper complexes (e.g. copper citrate, copper enolate) for bloom control in reservoirs and lakes. Care must be taken to treat the water supplies before a bloom is well established, however, because soluble cyanobacterial toxin concentrations, like those of the taste-and-odor compounds, increase substantially if the bloom is well developed. Bacterial degradation of the toxins in water supplies is usually slow process (Jones and Orr 1994). Intact cyanobacterial cells entering the treatment plant in large numbers may be removed by coagulation, flocculation, sedimentation and filtration but will lyse if oxidants are added first (Drikas et al., 2001a, 2002b). The soluble toxins cannot be removed by physical methods.

Water utilities that use rivers and large lakes as their raw water source usually cannot use copper-based algicides to control cyanobacterial blooms. Cyanobacterial control options in these cases are limited and usually are focused on controlling nutrient inputs from point discharges (notably secondary-treated wastewater discharges) and urban/agricultural runoff. When phosphorus is the growth-limiting nutrient, both external and internal (sediment) sources must be controlled. Internal nutrient cycling is usually prevented by in-lake treatments such as aeration.

Drinking Water Treatment

Hrudey et al. (1999), Yoo et al. (1995), and Drikas et al. (2001b) summarized effectiveness of water treatment processes for removing cyanobacterial cells and toxins from drinking water. All the evidence seems to suggest that activated carbon adsorption and oxidation are the most effective treatments for removing dissolved toxins, while routine coagulation, flocculation, and filtration and air flotation prior removes intact cells. The following summarize the available information.

Conventional coagulation, flocculation, sedimentation. Drikas et al. (2001a, 2001b) found that coagulation with iron salts and alum followed by conventional flocculation and sedimentation was effective for removing intact cyanobacterial cells from drinking water. However, they also found that rapid removal of sludge from basins was critical because the cells died in a short time, causing lysis and subsequent toxin release. Preoxidation should be discontinued if large numbers of cyanobacteria are entering the plant because the cells are killed, thus increasing the amount of toxin that has to be removed by other treatment procedures.

Membranes. Utrafiltration (UF) and microfiltration (MF) membranes effectively removed intact cyanobacterial cells from water without significantly damaging them, but MF membranes were harder to clean. Some toxin adsorption on UF membranes was observed by Drikas et al. (2001b). Soluble toxins generally are not effectively removed by either UF or MF membranes, though Hart and Stott (1993) found that microcystin spiked into raw water at 5 μg/L and 30 μg/L was removed to concentrations less than 1 μg/L by nanofiltration. Other information regarding cyanotoxin removal by membranes was presented by Hrudey et al. (1999).

Chlorination. Drikas et al. (2001b) found that chlorination was effective for destroying microcystin and cylindrospermopsin at pH < 8 if the dose was sufficient to provide a residual of 0.5 mg/L after 30-minutes contact. It was ineffective against anatoxin-a at pH 6-7. Chloramines were ineffective against any of the toxins because it they are not strong oxidants.

Ozone. Ozone effectively inactivates microcystin and anatoxin-a at dosages high enough to kill *Giardia* and *Cryptosporidium* if a residual is found after 1 minute contact. Cylindrospermopsin is probably destroyed, but more work is need before more definitive statements can be made. Saxitoxins were not effectively oxidized to low levels. Ozone effects on the individual toxins are described below (Drikas et al. 2001b):

Microcystins. Batch studies showed complete destruction of m-LR and m–LA in raw waters at O_3 dosages great enough to produce a residual after 5 minutes contact. Dosages ranged from 0.2 mg/L to 1.8 mg/L O_3. The dosages required to maintain a residual varied with the concentration of natural organic matter in the water supplies.

Anatoxin-a. Patterns of O_3 destruction of anatoxin-a were similar to those seen for the microcystin isomers. Higher initial O_3 dosages were required, however, and raged from 0.5 to 2.5 mg/L.

Saxitoxins. Six saxitoxins in a mixture were spiked into three Australia raw water samples and ozonated. Results indicated that the saxitoxins are not oxidized well by ozonation.

Other oxidants. Drikas et al. (2001b) evaluated the effectiveness of hydrogen peroxide and potassium permanganate ($KMnO_4$) in destroying m-LR. Hydrogen peroxide at a dose of 2 mg/L was ineffective, but 2 mg/L reduced the m-LR concentration by about 60% in 3 minutes and 95% in 10 minutes. In these studies, $KmnO_4$ was more effective than chlorine (75% removal in 10 minutes with 2.0 mg/L) for destroying soluble m-LR but not for lysing cells.

Chlorine dioxide (ClO_2), even though it is a strong oxidant, is ineffective at dosages typically used during water treatment. Hart and Stott (1993), cited by Hrudey et al. (1999) found that 6 mg/L ClO_2 reduced m-LR from 4.6 µg/L only to less than 1 µg/L, but a dose of 10 µg/L had no effect on about 4 µg/L intracellular toxin.

Powdered activated carbon (PAC). Most information about the effectiveness of PAC was derived from studies with m-LR, and, as is true in all studies involving activated carbon, the type of carbon and water-quality conditions were important variables.

Microcystins. Drikas et al. (2001b) reported that mesoporous carbons (i.e. those with a high percentage of pores in the 2-50 nm range) were the most effective for removing m-LR, but large differences in efficiency were observed in the adsorption of four microcystin

variants (5 µg/L concentration, 30 min contact) by two activated carbons (coal-based and wood-based). The order of effectiveness of both coal-based and wood-based carbon for adsorbing the four isomers was:

m-RR > m-YR > m-LR > m-LA

The coal-based PAC was more effective than the wood-based carbon in this study, but the authors stressed that different results can be expected with different carbons and raw-water quality conditions. The authors believed that m-LA could not be reliably removed by activated carbon adsorption.

Anatoxin-a. While removal of this cyanotoxin has been studied to some extent, Drikas et al. (2001b) believed that the data were insufficient for making definitive statements. However, they stated that PAC likely

Saxitoxins. Drikas et al. (2001b) found that the effectiveness of five PACs (carbon dosages 30 mg/L, 1 hour contact) for adsorbing saxitoxins in a mixture varying considerably and concluded that the relationship between molecular size of the toxins and the pore-volume distribution of the PAC was important.

Cylindrospermopsin. Drikas et al. (2001b) stated that no information regarding PAC effectiveness for removing saxitoxins is available in the peer-reviewed international literature, but they cited results of three studies in other references, two in Australian conference proceedings and one conducted by an activated carbon manufacturer. In one study "good" removals were observed after 30 minutes contact with a wood-based PAC at dosages less than 30 mg/L. In another study, a maximum 60% removal was achieved with a variety of PACs at dosages of approximately 6 mg/L and 30 min. contact. In the third study, 50% removal was achieved when by PAC at a concentration of only 2.7 mg/L; neither the PAC type nor contact time was mentioned.

Granular activated carbon (GAC). Drikas et al. (2001b) reported the results of pilot-scale studies of GAC effectiveness for adsorbing cyanotoxins from water at two water treatment plant. The results are summarized below:

Microcystins. Microcystin-LR and microcystin-LA (m-LA) were spiked into unchlorinated, treated water entering pilot GAC columns at two facilities. The first spike was added after 30 days operation. At one facility, both m-LR and m-LA were removed to levels below detection, but m-LA broke through at unacceptable levels at the second facility where the raw-water TOC was greater. Six months after startup, water entering the columns was again spiked with both isomers and opposite results were seen in that both m-LA and m-LR were removed to levels below detection at the second facility but broke through at the first. The explanation the authors gave was that microorganisms growing on the GAC at the first facility were unable to degrade either of the toxins but could degrade them at the second facility.

Microcystin-LR biodegradation was rapidly degraded in a reservoir after a bloom of *Microcystis aeruginosa* had been killed by copper sulfate, but only after a lag period of three days (Jones and Orr, 1994). The authors suggested that the lag period was required for the development of a microcystin-degrading population of microbes in the reservoir.

Drikas et al. (2001b) presented data that showing that a lag period of approximately 16 days was required for the establishment of a microcystin-degrading microbial population in laboratory-scale GAC columns. The GAC was taken from one of the pilot plants that had been in use for three months. The influent was spiked with 20 μg/L of m-LR and m-LA. During the first 16 days of operation, removal of both isomers was obviously by adsorption, not biodegradation, but thereafter nearly 100% of both isomers was removed. After 68 days, the GAC was removed from the columns, sterilized, and placed back in service. The influent was then spiked with 30 μg/L of both isomers, and breakthrough to high levels was immediate.

Anatoxin-a. Drikas et al. (2001b) cited the work of others wherein anatoxin-a adsorption data derived from small-scale GAC-column studies were used to predict removals by full-scale. . The investigators predicted that breakthrough would occur after about 15 weeks of operation, but removal by bacterial activity on the columns was not considered. While acclimation and biological degradation are likely to occur, the authors could not recommend GAC for removal of anatoxin-a.

Cylindrospermopsin. No data are presently available on which to base comments about GAC effectiveness for removing this cyanotoxin from water supplies.

Saxitoxins. Drikas et al. (2001b) studied saxitoxin removals by a single GAC brand (ACI) over a six month period. The influent to the laboratory-scale GAC column was spiked with a mixture of six saxitoxins at the beginning of the study, again one month later, and again six months after the study began. After six months, removals in terms of "saxitoxin toxicity equivalents" was still satisfactory, about 70%. The authors recommended that microporous carbons (pores < 2 nm), such as those made from good grade coal and coconut shells, should be used to remove saxitoxins.

Photolysis with ultraviolet light (UV). Drikas et al. (2001b) stated that conditions for toxin destruction by ultraviolet (UV) photolysis are likely to be outside the practical range for water treatment. They cited one study where irradiation of anatoxin-a at 254 nm was destroyed, but only at a dose twice that required for disinfection (about 30 mWs/cm^2).

Hrudey et al. (1999), citing others, reported conflicting results regarding the effectiveness of UV for cyanotoxin destruction studies. Rositano and Nicholson (1994) reported that UV alone and UV with hydrogen peroxide reduced m-LR levels by about 50% after 30 minutes, but Croll and Hart (1996) found that UV efficiently degraded m-LR and anatoxin-a at UV dosages of about 20,000 mWs/cm^2. Obviously, much more research is required before the true efficacy of UV irradiation for toxin destruction is known.

AWWA RESEARCH FOUNDATION STUDY OF CYANOTOXINS IN UNITED STATES WATER SUPPLIES

Carmichael (2001) analyzed cyanobacterial toxins in water samples submitted by utilities throughout the USA and Canada. Originally, 45 utilities agreed to submit samples over a two-year period at times when they were experiencing cyanobacterial blooms (i.e. > 2,000 cells/mL) in their water supplies. Ten "core utilities," collected samples twice a month for one year (December 1996 to December 1997). Participating utilities were selected on the basis of their geographic distribution within the USEPA regions; four Canadian utilities were included in the study as well.

Samples collected by the utilities included: (1) surface/subsurface/bloom in the water supply ("plankton net tow" or grab sample), (2) inlet to the treatment system (intake), (3) treatment plant inlet (plant influent), and (4) the plant outlet (finished water). Aliquots of samples (1), (2), and (3) were filtered through glass-fiber filters, and then the water samples and glass-fiber filters were sent to Wayne State University for analysis. Some utilities sent samples to Metropolitan Water District of Southern California (MWDSC) for Flavor Profile Analysis (FPA) and GC/MS analyses of geosmin (earthy smell) and 2-methylisoborneol (2-MIB, musty smell). Each utility was asked to provide a map of their reservoir showing sampling locations.

The project was completed in January, 1998, and 677 water samples and 458 filters had been analyzed for microcystins by ELISA and PPIA. A total microcystin concentration (ng/mL) was obtained by adding the concentrations of soluble microcystins in the filtrates and the concentrations extracted from cells retained on the filters. The results were as follows:

- Total water samples: 677
- Total filter-paper samples: 458
- Total ELISA assays: 1135 at Wright State University, 1135 at the University of Wisconsin.
- Total PPIA assays: 1135 at Wright State University and 275 at University of Wisconsin
- Samples > 1 μg/L microcystins:
  - -Source water: 12 (1.8%)
  - -Intake water: 8 (1.2%)
  - -Influent water: 7 (1.0%)
  - -Finished water: 2 (0.3%)

Microcystins were found in 80% of the 677 samples analyzed by ELISA, but only 4.3% of the samples contained levels that exceeded the WHO guideline (1 μg/L). Only two finished-water samples contained microcystins at levels greater than the 1 μg/L guideline.

All the available data indicate that the treatment processes at the treatment plants during the period of this study effectively reduced microcystin concentrations to safe levels and that the majority of the water supplies with cyanobacteria did contain microcystins. Of the 243 samples evaluated for taste and odor, 75% were positive for either taste or odor by FPA or the presence of either geosmin or 2-MIB, and 82% of the 148 samples that were positive for taste or odor also contained microcystins. Carmichael recommended that utilities purchase commercially available kits for monitoring cyanotoxins in their water supplies.

SUMMARY

Toxic cyanobacteria (blue-green algae) are the latest in a series microbial organisms that can potentially pose some level of health risk in public water supplies. In the past, cyanobacteria have been regarded only as nuisance organisms that cause taste-and-odor problems in drinking water, but their potential for producing toxins with human health effects has been highlighted by their inclusion in USEPA's 1998 Candidate Contaminant List, which specifies substances and microorganism in water that should be targeted for further investigation.

The cyanotoxins of most concern in the United States are the hepatotoxins (primarily the microcystin isomers microcystin-LA and -LR) and the neurotoxins (mainly anatoxin-a and anatoxin-a(S)). Other neurotoxins include the saxitoxins, the best known of which cause red tide in costal waters, but even though they have been found in blooms of several *Anabaena* species, they are of less concern in the United States than anatoxin-a and the hepatotoxins. Blooms of *Cylindrospermum raciborskii,* which produce the cytotoxic (hepatoxic) alkaloid cylindrospermopsin, appeared recently in Florida, evoking considerable public interest but producing no health threat. Other individual species of *Cylindrospermum* have been shown to produce anatoxin-a. Cylindrospermopsin has also been given top priority for future study.

Worldwide, the microcystins, which are produced primarily by *Microcystis aeruginosa* and certain *Anabaena* and *Oscillatoria* species, are likely to be present in eutrophic and hypereutrophic water supplies at concentrations sufficiently high to cause concern. These compounds have been shown to be liver tumor promoters, an issue that concerns public health officials in other countries. A special panel convened by the USEPA to advise the agency on cyanotoxin priorities for future study recommended that highest priority be assigned to the hepatotoxic microcystins, the cytotoxic alkaloid cylindrospermopsin, which also affects the liver, and the neurotoxic alkaloid anatoxin-a. Medium-to-high priority was assigned to the neurotoxic saxitoxins and anatoxin-a(s), some of which are produced by blue-green algae. The panel determined that the lipopolysaccharide toxins, which are skin- and mucous membrane irritants, are not enough of a problem in the United States to warrant concern at this time.

The World Health Organization (WHO) has established a drinking water guideline of 1.0 μg/L for m-LR, which is the most prevalent of the microcystins. Australia probably leads the world in cyanobacterial toxin research and in setting guidelines for many of the cyanotoxins, but no guideline has been established for any of the cyanotoxins in the United States.

Microcystins-LR, which is the most-common cyanotoxin in blooms, can be removed effectively from drinking water by treatment with oxidation with ozone, chlorine, and permanganate and adsorption by both PAC and GAC. Neither UV irradiation, chlorine dioxide, nor chloramines are effective agents for destroying cyanotoxins. The effectiveness of PAC and GAC is water-quality specific, carbon specific, and toxin specific. For example, microcystin-LA, a less-common microcystin isomer, was not effectively removed by GAC in studies at the Australian Water Research Centre. Usually, carbons with a high percentage of large pores (2-50 nm) are best except for saxitoxins, which are better removed by microporous carbons.

Care should be taken to minimize the entry of cyanobacterial cells into the treatment plant because they likely will be lysed during treatment and increase the levels of soluble toxins. Also, if copper sulfate is used to eliminate cyanobacteria in lakes and reservoirs, it should be applied before a bloom is established to avoid lysing the cells and subsequent high levels of soluble toxins in the influent to the water treatment plant. Carmichael's AWWA Research Foundation project involving 24 utilities in the United States and Canada demonstrated that water treatment plants in the United States and Canada are generally able to reduce microcystin levels to below the WHO guideline of 1 µg/L.

Easy-to-use kits for detecting microcystins in water supplies have been developed and are commercially available. Kits for other toxins are presently under development developed. Utilities experiencing problems with cyanobacteria each year should consider purchasing these reasonably inexpensive kits and monitor toxin levels in their raw and finished water during critical periods.

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