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## Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems

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This fact sheet provides public water suppliers basic information on human health effects, analytical screening tools, and the effectiveness of various treatment processes to remove or inactivate the three most important cyanotoxins that can occur in the waters of almost every part of the US and are listed on the third Candidate Contaminant List (CCL): microcystin-LR, anatoxin-a, and cylindrospermopsin. Other cyanotoxins such as saxitoxins and anatoxin-a(S) also occur in US waters, but they are generally thought to be less common. Therefore, this fact sheet does not address these other well known toxins produced by cyanobacteria such as the paralytic shellfish toxins (Saxitoxin family), anatoxin-a(S), the lyngbyatoxins, or taste and odor contaminants caused by the cyanobacteria.

### Background

The Safe Drinking Water Act (SDWA) protects public health by regulating the nation's public drinking water supply and its sources: rivers, lakes, reservoirs, springs, and ground water wells. The SDWA requires the U.S. Environmental Protection Agency (EPA) to publish a list of unregulated contaminants that are known or expected to occur in public water systems in the US that may pose a risk in drinking water. This list is known as the Contaminant Candidate List (CCL). For more information on the CCL program go to <http://water.epa.gov/scitech/drinkingwater/dws/ccl/>

The cyanotoxins included in the most recent CCL are produced by several species of cyanobacteria (cyanobacteria are known as blue-green algae). The most widespread of the cyanotoxins are the peptide toxins in the class called microcystins. There are at least 80 known microcystins, including Microcystin-LR, which is generally considered one of the most toxic. More than a dozen countries (including Canada, Brazil, New Zealand, and Australia) have developed regulations or guidelines for microcystins in drinking water and recreational waters. Most of the drinking water guidelines are based on the World Health Organization provisional value for drinking waters of 1.0 µg/L microcystin-LR. No federal regulatory guidelines for cyanobacteria or their toxins in drinking water or recreational waters exist at this time in the United States.

### Causes of cyanobacteria harmful algal blooms

Cyanobacteria are photosynthetic bacteria that share some properties with algae and are found naturally in lakes, streams, ponds, and other surface waters. Similar to other types of algae, when conditions are favorable, cyanobacteria can rapidly multiply in surface water and cause "blooms." The conditions that enhance the growth of cyanobacteria are described below. Several types of cyanobacteria, like for example *Anabaena flos-aquae*, have gas-filled cavities that allow them to float to the surface or to different levels below the surface, depending on light conditions and nutrient levels. This causes the cyanobacteria to concentrate on the water surface, causing

the typical pea-soup green color or blue-green "scum." Some cyanobacteria like *Planktothrix agardhii*, can be found in bottom sediments and float to the surface when mobilized by storm events and other sediment disturbances. Other cyanobacteria blooms may remain dispersed through the water column (*Cylindrospermopsis sp.*) leading to a generalized discoloration of the water.

Factors that affect cyanobacterial bloom formation and persistence include light intensity and total sunlight duration, nutrient availability (especially phosphorus), water temperature, pH, an increase in precipitation events, water flow (whether water is calm or fast-flowing), and water column stability. Although bloom conditions in much of the US are more favorable during the late summer, the interrelationship of these factors causes large seasonal and year-to-year fluctuations in the cyanobacteria levels. Some toxin-producing strains can occur early in the summer season while others are only found during late summer.

Cyanobacterial blooms can be harmful to the environment, animals, and human health. The bloom decay consumes oxygen, creating hypoxic conditions which result in plant and animal die-off. Under favorable conditions of light and nutrients, some species of cyanobacteria produce toxic secondary metabolites, known as cyanotoxins. Common toxin-producing cyanobacteria are listed in Table 1. The conditions that cause cyanobacteria to produce cyanotoxins are not well understood. Some species with the ability to produce toxins may not produce it under all conditions. These species are often members of the common bloom-forming genera. Both non-toxic and toxic varieties of most of the common toxin-producing cyanobacteria exist, and it is impossible to tell if a species is toxic or not toxic by looking at it. Also, even when toxin-producing cyanobacteria are present, they may not actually produce toxins. Furthermore, some species of cyanobacteria can produce multiple types and variants of cyanotoxins. Molecular tests are available to determine if the cyanobacteria, *Microcystis* for example, carry the toxin gene; quantitative cyanotoxin analysis is needed to determine if the cyanobacteria are actually producing the toxin. Water contaminated with cyanobacteria can occur without associated taste and odor problems.

In most cases, the cyanobacteria toxins exist intracellularly in the cytoplasm and are retained within the cell. Anatoxin-a and the microcystin variants are found intracellularly approximately 95% of the time during the growth stage of the bloom. For those species, when the cell dies or breaks, the cell membrane ruptures and the toxins are released into the water (extracellular toxins). However, in other species, cylindrospermopsin for example, a significant amount of the toxin may be naturally released to the water by the live cyanobacterial cell; the reported ratio is about 50% intracellular and 50% extracellular. Extracellular toxins may absorb to clays and organic material in the water column and are generally more difficult to remove than the intracellular toxins.

### **Health effects caused by cyanotoxins**

The cyanotoxins include neurotoxins (affect the nervous system), hepatotoxins (affect the liver), and dermatotoxins (affect the skin). The presence of high levels of cyanotoxins in recreational water and drinking water may cause a wide range of symptoms in humans (Table 1) including fever, headaches, muscle and joint pain, blisters, stomach cramps, diarrhea, vomiting, mouth ulcers, and allergic reactions. Such effects can occur within minutes to days after exposure. In severe cases, seizures, liver failure, respiratory arrest, and (rarely) death may occur. There is

**Table 1. Cyanotoxins on the Contaminant Candidate List (CCL)**

<b>Cyanotoxin</b>	<b>Number of known variants or analogues</b>	<b>Primary organ affected</b>	<b>Health Effects<sup>1</sup></b>	<b>Most common Cyanobacteria producing toxin<sup>2</sup></b>
Microcystin-LR	80~90	Liver	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage	<i>Microcystis</i> <i>Anabaena</i> <i>Planktothrix</i> <i>Anabaenopsis</i> <i>Aphanizomenon</i>
Cylindrospermopsin	3	Liver	Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion	<i>Cylindrospermopsis</i> <i>Aphanizomenon</i> <i>Anabaena</i> <i>Lyngbya</i> <i>Rhaphidiopsis</i> <i>Umezakia</i>
Anatoxin-a group <sup>3</sup>	2-6	Nervous System	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death	<i>Anabaena</i> <i>Planktothrix</i> <i>Aphanizomenon</i> <i>Cylindrospermopsis</i> <i>Oscillatoria</i>

<sup>1</sup>Source: *Harmful Algal Research and Response National Environmental Science Strategy (HARRNESS)*

<sup>2</sup> Not all species of the listed genera produce toxin; in addition, listed genera are not equally as important in producing cyanotoxins.

<sup>3</sup>The anatoxin-a group does not include the organophosphate toxin anatoxin-a(S) as it is a separate group. In the US, the most common member is thought to be anatoxin-a, and thus this toxin is listed specifically.

evidence that long-term exposure to microcystins and cylindrospermopsin may promote the growth of tumors and may cause cancer.

There have been many documented reports of dog, bird and livestock deaths throughout the world as the result of consumption of surface water with cyanobacterial blooms. Infrequently, human deaths have also been documented. Patients exposed intravenously to water containing microcystins in a kidney dialysis center in Brazil in 1996 resulted in 50 deaths. The actual risk to cyanotoxins at low levels in drinking water and the long-term effects to the exposure to these toxins is uncertain.

### Screening Methods

Table 2 describes the methods available for cyanotoxin measurement in freshwater. There are commercially field test kits available for use as a screening tool to determine the presence or absence of specific cyanotoxins in a water supply. Screening kits (ELISA kits) are available for the microcystins, cylindrospermopsin and saxitoxin, but are not currently available for anatoxin-a. Although they provide rapid results, generally these kits have limitations in accuracy, sensitivity, and specificity. If the screening test is positive, the water system should send samples to a laboratory capable of measuring the concentrations of specific

**Table 2. Methods Available for Cyanotoxin Detection \***

<b>Freshwater Cyanotoxins</b>			
<b>Methods</b>	<b>Anatoxins</b>	<b>Cylindrospermopsins</b>	<b>Microcystins</b>
<b>Biological Assays</b>			
Mouse	Yes	Yes	Yes
Protein Phosphatase Inhibition Assays (PPIA)	No	No	Yes
Neurochemical	Yes	No	No
Enzyme-Linked Immunosorbent Assays (ELISA)	In progress	Yes	Yes
<b>Chromatographic Methods</b>			
<b>Gas Chromatography</b>			
Gas Chromatography with Flame Ionization Detection (GC/FID)	Yes	No	No
Gas Chromatography with Mass Spectrometry (GC/MS)	Yes	No	No
<b>Liquid Chromatography</b>			
Liquid Chromatography / Ultraviolet-Visible Detection (LC/UV or HPLC)	Yes	Yes	Yes
Liquid Chromatography/Fluorescence (LC/FL)	Yes	No	No
<b>Liquid Chromatography combined with mass spectrometry</b>			
Liquid Chromatography Ion Trap Mass Spectrometry (LC/IT MS)	Yes	Yes	Yes
Liquid Chromatography Time-of-Flight Mass Spectrometry (LC/TOF MS)	Yes	Yes	Yes
Liquid Chromatography Single Quadrupole Mass Spectrometry (LC/MS)	Yes	Yes	Yes
Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/MS/MS)	Yes	Yes	Yes

\*Adapted from [Analytical Methods for Cyanotoxin Detection and Impacts on Data Interpretation](#), presentation by Keith Loftin, Jennifer Graham, Barry Rosen (U.S. Geological Survey) and Ann St. Amand (Phycotech) at the 2010 National Water Quality Monitoring Conference, Workshop. Guidelines for Design, Sampling, Analysis and Interpretation for Cyanobacterial Toxin Studies at Denver, CO on April 26, 2010.

cyanotoxins using more accurate techniques such as Liquid Chromatography combined with Mass Spectrometry (LC/MS).

### **Cyanotoxin Treatment and Bloom Management**

Once cyanobacteria and/or their cyanotoxins are detected in the surface water supplying the water system, the treatment system operators can act to remove or inactivate them in a number of ways. Some treatment options are effective for some cyanotoxins, but not for others. Therefore, drinking water operators must know the growth patterns and species of cyanobacteria that dominates the bloom, the properties of the cyanotoxins (i.e., intracellular or extracellular), and the most effective treatment process. Applying the wrong treatment process at a specific state in treatment could damage cells and result in the release rather than removal of cyanotoxins.

Table 2 summarizes the effectiveness of different types of water treatment to remove intact cyanobacteria cells and treatment processes that are effective in removing extracellular dissolved toxins of several of the most important cyanobacteria. Drinking water operators are encouraged to monitor the treated water to guarantee the removal of cyanotoxins.

To avoid the release of cyanotoxins into the water, drinking water operators can undertake different management strategies to deal with cyanobacteria blooms. For example, those drinking water utilities that have access to more than one source of water supply, an alternative is to change sources. Another management alternative is to adjust intake depth to avoid drawing contaminated water and cells into the treatment plant.

Pretreatment oxidation at the intake, although often used to reduce taste and odor compounds and to reduce zebra mussels and to reduce other contaminants, poses several concerns with respect to breaking cells and releasing toxins. Copper sulfate and ozone at the intake removes the algal bloom, but is not recommended because of the risk of lysing algal cells. Chlorination, in addition to breaking the cells, has the potential to produce disinfection by-products during water treatment. If pretreatment oxidation is needed, potassium permanganate ( $\text{KMnO}_4$ ) has been demonstrated to be effective in removing *Microcystis* cells with no release of toxin. It is recommended that powdered activated carbon (PAC) be used in addition to remove any toxins that may have been released.

The standard drinking water treatment processes (coagulation, flocculation, sedimentation and filtration), have shown to be effective in removing intracellular cyanotoxins. Coagulation, flocculation and dissolved air flotation (DAF), are more effective than sedimentation. Microfiltration and ultrafiltration are highly effective at removing intact cyanobacterial cells. When a bloom occurs and cells are carried through to the filters, backwash should be more frequent to reduce the risk of toxins release in the water.

For removal of extracellular toxins, drinking water operators may use activated carbon, membrane filtration and chemical inactivation (Ultraviolet (UV), disinfectants and oxidants). Both powdered activated carbon (PAC) and granular activated carbon (GAC) have been effective in absorbing microcystin and cylindrospermopsin, although microcystin variants may have different adsorption efficiencies. The performance of activated carbon depends on the concentration of the toxin and the dose and origin of the activated carbon. Jar tests are recommended to test the effectiveness of various PAC types, with the implementation of the carbon with the greatest capacity for removal of the target contaminants. GAC filters are effective in removing microcystins if they are properly replaced or regenerated when total organic carbon breakthrough is high. Usually, higher concentrations of activated carbon are necessary to effectively remove toxins; repeated treatment may be needed to totally remove the toxins completely. Nanofiltration and reverse osmosis are effective in removing cylindrospermopsin and microcystin. However, site specific tests are recommended as removal efficiency depends on the membrane pore size distribution and water quality.

Ultraviolet (UV) treatment is effective in destroying microcystin, anatoxin-a, and cylindrospermopsin cells but higher doses than are practicable are required, making it a not viable treatment. UV has been used along with a catalyst (titanium dioxide) to oxidatively decompose the toxins. However, the effectiveness of this process is largely dependent on the

**Table 2. Cyanotoxin Treatment Processes and Relative Effectiveness**

Treatment Process	Relative Effectiveness
<b><i>Intracellular Cyanotoxins Removal (Intact Cells)</i></b>	
Pretreatment oxidation	Avoid pre-oxidation because often lyses cyanobacteria cells releasing the cyanotoxin to the water column.
Coagulation/Sedimentation/ Filtration	Effective for the removal of intracellular toxins when cells accumulated in sludge are isolated from the plant and the sludge is not returned to the supply after sludge separation.
Membranes	Study data is scarce; it is assumed that membranes would be effective for removal of intracellular cyanotoxins. Microfiltration and ultrafiltration are effective when cells are not allowed to accumulate on membranes for long periods of time.
Flotation	Flotation processes, such as Dissolved Air Flotation (DAF), are effective for removal of intracellular cyanotoxins since many of the toxin-forming cyanobacteria are buoyant.
Oxidation processes	Avoid because often lyses cyanobacteria cells releasing the cyanotoxin to the water column.
<b><i>Extracellular Cyanotoxins Removal</i></b>	
Membranes	Depends on the material, membrane pore size distribution, and water quality. Nanofiltration and ultrafiltration are likely effective in removing extracellular microcystin. Reverse osmosis filtration would likely only be applicable for removal of some extracellular cyanotoxins like cylindrospermopsin. Cell lysis is highly likely. Further research is needed to characterize performance.
Potassium Permanganate	Effective for oxidizing microcystins and anatoxins. Further research is needed for cylindrospermopsin.
Ozone	Very effective for oxidizing extracellular microcystin, anatoxin-a and cylindrospermopsin.
Chloramines	Not effective
Chlorine dioxide	Not effective with doses used in drinking water treatment.
Chlorination	Effective for oxidizing extracellular cyanotoxins as long as the pH is below 8, ineffective for anatoxin-a.
UV Radiation	Effective of degrading microcystin and cylindrospermopsin but at impractically high doses.
Activated Carbon	PAC: Most types are generally effective for removal of microcystin, anatoxin-a and cylindrospermopsin, especially wood-based activated carbon. GAC: Effective for microcystin but less effective for anatoxin-a and cylindrospermopsins.

organic content of the water. Oxidants like chlorine, ozone and  $\text{KMnO}_4$  are effective mechanisms for inactivation of microcystin but chlorine effectiveness is pH-dependent. Different cyanotoxins react differently to chlorine, for example, it has proved to be ineffective for the inactivation of anatoxin-a. However, when pH is below 8, chlorine is effective for inactivation of microcystin and cylindrospermopsin. Ozone is a good oxidant of microcystins, anatoxin-a and cylindrospermopsin, but its efficacy is also pH-dependent and may be affected by the presence of organic matter. Although ozone is pH-independent for the oxidation of microcystin, is pH-dependent for the oxidation of anatoxin-a (pH 7 to 10) and for cylindrospermopsin (4 and 10).  $\text{KMnO}_4$  is effective in oxidizing microcystin and anatoxin-a (from pH 6 to 8), but is not very reactive with cylindrospermopsin. Chloramines and chlorine dioxide are not effective treatments for microcystin, anatoxin-a and cylindrospermopsin.

Formation of disinfection by-products is another potential problem with the use of ozone, copper sulfate, and chlorine when there are high bromide concentrations in the water. However, results from studies on the impact of chlorination of cell-bound toxins and resulting disinfection by-products formation are contradictory. The majority of the findings suggest that pre-chlorination should ideally be avoided during blooms, unless adequate CT values can be guaranteed to ensure efficient oxidation of broken cyanobacteria.

### **Developing a Contingency Plan**

Water supply managers should develop a contingency plan when cyanobacterial blooms may occur. Most algal blooms are not toxic, and the plan should address how to determine the risk associated with each event. Elements of such a plan should include determining when and where to sample; sampling frequency; sample volume; whether to sample for cyanobacterial cells or specific cyanotoxins or both; which analytical screening test to use; and conditions when it is necessary to send sample(s) to an identified laboratory for confirmation. Drinking water system operators should also develop alternative plans for treatment before results are known; including what treatment option(s) to use if test results are positive; and identifying a process for public notification. Chapter 6 (Situation Assessment, Planning and Management) from the WHO's *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management* could be used as a resource to develop such plans.

### **Where can I get more information?**

For more information, please visit the Cyanobacteria Harmful Algal Blooms (CyanoHABs) web page at <http://www.epa.gov/nandppolicy/links.html#hab> .

### **Acknowledgements**

The author gratefully acknowledges the valuable contribution from Dr. Judy Westrick, Lake Superior State University, in developing this work.

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