

Advancing the Science of Water Managing Algal Toxins

Blue-green algae, also known as cyanobacteria, are commonly found in fresh water bodies and are widely recognized as sources of tastes and odors and algal toxins (cyanotoxins) in water supplies. Cyanobacteria are a normal component of the natural biota and tolerate a wide range of climatic conditions and environments. A rise in the number of cyanobacterial blooms, caused by eutrophication from decaying plant materials and man-made pollution, is resulting in more taste-and-odor compounds and natural toxins being produced, demanding the attention of water treatment authorities. While not as common as taste and odor compounds, because of their high toxicity algal toxins are a much greater concern when present in drinking water. Due to global climate change, toxin-producing cyanobacteria are spreading into more temperate regions and becoming a more widespread problem.

Currently, there are no Environmental Protection Agency regulations for cyanotoxins. However, three cyanotoxins are included in final contaminant candidate list (CCL3): Anatoxin-a, Microcystin-LR, and Cylindrospermopsin. Currently the only cyanobacterial toxin class that has been internationally assessed for health risk is the microcystins. The World Health Organization (WHO) has issued a provisional guideline value of 1 microgram per liter ($\mu\text{g/L}$) for microcystin-LR and many countries have developed their own guidelines, depending on the types of algal toxins found in their source waters.

Due to the increase in cyanobacterial blooms, the occurrence of a number of toxic metabolites (i.e., algal toxins) in water supplies has also increased. There is growing concern about the potential for negative health effects in humans and animals due to these toxins. These toxins enter water supplies through natural production and metabolic activities, and also through cell lysis and subsequent release of toxins. Water collection and treatment activities may contribute to the release of algal toxins.

Presently, about 30 species of cyanobacteria are known; however, not all produce toxins. The organisms most frequently associated with toxin production are *Microcystis*, *Oscillatoria*, *Cylindrospermopsis*, *Anabaena*, *Planktothrix*, *Aphanizomenon*, *Nodularia*, and *Lyngbya*.

Most poisoning by the cyanobacteria listed above involves three types of toxins (specific toxic compounds are listed in parentheses): (1) hepatoxins (microcystin [usually microcystin-LR and microcystin-LA], cylindrospermopsin, and nodularian), which are taken up by the liver and cause weakness and anorexia; (2) neurotoxins (usually anatoxin and saxitoxin), which effect the nervous system; and (3) dermatotoxins (aplysiatoxin and lyngbyatoxin), which cause skin and mucous irritations upon contact.

Taste-and-Odor Compounds and Toxic Algae

The most frequently cited cyanobacterial metabolites are geosmin and 2-methylisoborneol (MIB). Geosmin and MIB impart objectionable earthy/musty odors to the water. Attempts to use taste-and-odor parameters as potential surrogates for the presence of toxins have been found to be inconclusive. Just as it is true that most of the cyanobacterial species do not cause taste-and-odor problems, it also is true that most do not produce toxins. Some species that produce taste-and-odor compounds, however, can also produce toxins.

The Water Research Foundation and Algal Toxin Research

The Water Research Foundation has sponsored research on toxic algae since 1993, initially producing a comprehensive resource guide for utilities: [*Cyanobacterial \(Blue-Green Algal\) Toxins: A Resource Guide*](#) (order #90693). Since the publication of the guide, The Foundation has funded additional research on control, treatment, and detection methods for algal toxins, much of it in collaboration with research partners.

The increased frequency of cyanobacterial blooms in the United States prompted The Foundation, in partnership with the U.S. Environmental Protection Agency, to fund one of the first projects to investigate algal toxins as a potential threat to United States water systems. The comprehensive study, published in 2001 as [*Assessment of Blue-Green Algal Toxins in Raw and Finished Drinking Water*](#) (order #90815), assessed microcystin occurrence and treatment removal capabilities. During the project, 45 utilities in the United States and Canada were surveyed for two years during algal blooms. Microcystin was found in 80% of the source waters, and 4.3% of the samples were above the WHO guidelines (1µg/L). The study also showed that almost all utilities had adequate procedures to reduce microcystin to safe levels in finished water.

Treatment

There are several treatment options—conventional and advanced—available for the removal of algal toxins. The key is in understanding the specific toxin of concern, because different toxins are removed/inactivated at varying degrees by different treatment technologies. For example, several research studies indicate that water treatment plants that meet Stage 1 and 2 of the Disinfectants/Disinfection By-product Rule by using ozone have a considerable level of protection from several types of algal toxins such as microcystin, but not from saxitoxin. On the other hand, pre-oxidants such as potassium permanganate, ozone, and chlorine (which are used to mitigate cyanobacteria) have been found to lyse cyanobacteria, which releases toxins; therefore, it is recommended that coagulation be used prior to oxidation to remove whole cells.

One of first Foundation projects addressing removal of algal toxins through water treatment was conducted as a Tailored Collaboration project with United Water International (Australia). The report, [*Removal of Algal Toxins from Drinking Water Using Ozone and GAC*](#) (order #90904), was published in 2002. The researchers conducted lab and pilot plant tests for the control of algal toxins through treatment (ozone, granular activated carbon [GAC], biological filtration) to assess the optimal conditions under which microcystin, anatoxin-a, and

saxitoxin are inactivated. It was found that ozone is an efficient treatment for anatoxin-a and microcystin but saxitoxin is not readily destroyed under the same conditions. The study also determined that GAC adsorption is not effective for the removal of microcystin toxins; however, when the GAC is used in the biological mode, removal is excellent. Effective removal of toxicity was found with GAC for saxitoxin. Biological filtration was not effective for saxitoxin.

Building on this project as well as other research, an ongoing Tailored Collaboration project with the City of Cocoa (Fla.), [**Treatability of Algal Toxins Using Oxidation, Adsorption, and Membrane Technologies**](#) (order #2839), was funded to identify and assess viable control and treatment methods, including design, operating criteria, and estimated treatment costs, to mitigate microcystin-LR in finished water. The study conducted bench-scale tests for the following treatment technologies: UV/H₂O₂, ozone/AOP, powdered activated carbon (PAC), GAC, biological degradation, and membranes (reverse osmosis [RO] and nanofiltration [NF]). UV/H₂O₂ was effective but was dictated by H₂O₂ concentrations and availability; UV alone was not effective. The study found GAC was effective at removing microcystin when GAC was replaced frequently and total organic carbon concentrations were low. The ozone/AOP combination was effective at removal, mostly if pH was below 7; doses as low as 0.4 mg/L achieved microcystin-LR removal greater than 97%. PAC was also able to remove microcystin-LR at doses of 10 mg/L and contact time of 30 minutes. Biological degradation provided 35% removal of microcystin-LR; thus, it was recommended that it should be used as a polishing step in conjunction with other treatment methods, such as UV, oxidation, ozonation, PAC, and GAC. The RO and NF membranes tested removed microcystin-LR efficiently, at a minimum of 95%. Based on the effectiveness of the technologies above, costs of implementation and engineering considerations are provided in the report for utilities to make suitable treatment decisions.

Recently, the project [**“Evaluation of Integrated Membranes for Taste and Odor and Algal Toxin Control”**](#) (project #4016) was initiated with the Australian Cooperative Research Center for Water Quality and Treatment (CRCWQT) to study the feasibility of membrane technologies (ultrafiltration [UF], NF, and RO) in conjunction with coagulation, PAC, or microfiltration (MF) membranes for the removal of taste-and-odor compounds and algal toxins. The study will focus on the removal of extracellular MIB, geosmin, cylindrospermopsin, and the major microcystin variants by RO and NF. It will also examine the development of a UF integrated membrane system ideally suited to the removal of dissolved algal metabolites (microcystins, geosmin, MIB, and cylindrospermopsin). The removal of cyanobacteria during integrated membrane treatment and the subsequent potential release of algal metabolites (i.e., toxins) will also be evaluated. Lastly, the project will provide recommendations for the selection of membrane systems that will meet requirements for removal of algal metabolites.

Detection Methods

In response to the increasing frequency of cyanobacterial blooms, greater awareness of toxic cyanobacteria, and new methods of detecting and monitoring cyanotoxins, robust analytical methods must be available to monitor for toxins and assess their significance. Methods for algal toxins either detect toxins or measure toxicity. There are several detection methods for algal

toxins: high performance liquid chromatography (HPLC), gas chromatography coupled with mass spectrometry (GC/MS), liquid chromatography coupled with mass spectrometry or tandem mass spectrometry (LC/MS and LC/MS/MS), and enzyme-linked immunosorbent assay (ELISA). The toxicity assays include the neuroblastoma assay and the phosphatase inhibition (PIP) assays. The ELISA and PIP are currently commercially available. Depending on the algal toxin compound, one method may be preferable over another. More recently, molecular methods have been developed to identify the genes controlling toxin production.

One of the most critical tools for the analysis of contaminants in water is sampling. The recently funded Foundation project #4212, [**“Rapid Concentration and Detection of Microcystin and Other Cyanobacterial By-Products in Drinking Water,”**](#) will utilize molecularly imprinted polymers (MIPs) and surface enhanced Raman spectroscopy (SERS) to concentrate and quantify cyanobacterial byproducts. The goal of the research is to develop a simple and economically feasible detection scheme for microcystin LR and MIB that could be implemented in a water treatment facility.

A report published in 2007, [***Determination and Significance of Emerging Algal Toxins***](#) (order #91170), co-sponsored with the CRCWQT, evaluated and developed methods available for detection of algal toxins. The study further developed methods for the determination of saxitoxin, anatoxin-a, and cylindrospermopsin. This included their determination in a single method: an LC/MS/MS method for determining saxitoxin, anatoxin-a, and cylindrospermopsin in a single analytical run was successfully developed. (For low concentrations, pre-concentration using carbon-based solid phase extraction cartridges was required.) The neuroblastoma assay detected saxitoxin that was not detected by other methods. ELISA methods, including commercially available test kits, were also evaluated for determining microcystin, with good results. Gene probes for the detection of toxic cyanobacteria were applied to a number of field samples, but it was found that determination of cyanobacterial toxicity using gene probes needed further validation using a wider range of samples. Overall, the project suggests HPLC methods and to a lesser extent ELISA can detect and quantitate toxins present at low $\mu\text{g/L}$ levels. Finally, an occurrence study detected microcystin in both United States and Australian raw water samples. Cylindrospermopsin was detected in Australian but not United States samples. Saxitoxin and anatoxin-a were not detected in any raw water samples in either country.

Several Foundation projects researching the genetic basis for toxin production by cyanobacteria have been funded in the past few years. As most methods to detect toxins only work after the bloom has occurred, molecular tools, which can determine the potential of a population to produce toxins, may provide the best forecasting tool available for source water control strategies. Molecular technology enables spatial and temporal information on the distribution of toxic algae to be gathered rapidly with replication. These key advantages are not available when using microscopic analysis and provide an important augmentation to routine microscopy and toxin analysis when more detailed and rapid information about key toxic species is required.

A 2007 report, [***Development of Molecular Reporters for Monitoring Microcystis Activity and Toxicity***](#) (order #2818, electronic format only), identified regions of the genome of *Microcystis aeruginosa* that could be used as molecular targets in the rapid identification of

potentially toxic algal blooms containing these cyanobacteria. This project developed a presence/absence approach (multiplex- polymerase chain reaction [PCR]), as well as a quantitative approach (using qPCR), which could be easily applied in field situations. To demonstrate that these molecular tools worked under field conditions, the researchers tested the probes during blooms of potentially toxic cyanobacteria *Microcystis* spp., which persisted in western Lake Erie in the United States during August 2002–2004, when microcystin concentrations exceeded the safety limit set by WHO. The presence of *Microcystis* spp. in water samples was confirmed through the multiplex PCR reaction using a combination of four primer sets. Quantification of *Microcystis* was achieved using the real-time PCR assay.

Another molecular-based detection method project, [Early Detection of Cyanobacterial Toxins Using Genetic Methods](#) (order #91198), developed a rapid genetic method to identify toxic cyanobacteria. A comprehensive literature review and industry questionnaire were used to identify and select a suitable platform technology for rapid genetic identification of toxic cyanobacteria. The project, co-sponsored with the CRCWQT, identified the genes likely to be involved in cylindrospermopsin production in *C. raciborskii*, and attempts were made to identify and sequence genes likely to be involved in the production of anatoxin-a. The project also developed a simple and rapid method for the preparation of cyanobacteria-containing water samples. Finally, a method was developed and tested successfully in the laboratory and the field to adapt conventional PCR assays for cylindrospermopsin-producing cyanobacteria to real-time PCR.

An ongoing study, [Methods for Measuring Toxins in Finished Water](#) (project #4020), is investigating a range of biological methods, given that they are becoming more prevalent, as a tool for source water and finished water toxicity measurements that may be suitable for detecting toxins in finished waters. Biological assays have not been refined for application to drinking water. One of the key objectives of the project, co-sponsored with the Drinking Water Inspectorate (U.K.) and CRCWQT, is to define methods (i.e., MicroTox[®] and CheckLight) for quenching chlorine in finished water, as this is known to interfere with current toxicity screening methods. It is expected that these assays will be capable of implementation in a routine process monitoring program during production and delivery of finished drinking water.

Because none of the aforementioned detection methods are standardized, The Foundation funded an ongoing research project with the USEPA, [Criteria for Quality Control Protocols for Various Algal Toxin Methods](#) (project #2942), to develop quality assurance protocols for the quantitative analysis of microcystin, saxitoxin, cylindrospermopsin, and anatoxin-a present in water and of cyanobacterial extracts. The project report, which is expected to be published in 2010, will recommend extraction methods, concentration methods, production of analytical standards, and preservation of water samples containing toxins of the various algal toxins.

Source Water Control

Algal blooms occur seasonally and are generally a result of over-enrichment by plant nutrients, particularly nitrogen and phosphorous. Human influences such as urbanization, increasing population, and agriculture contribute to the cause of algal blooms. As stated previously, not all algal blooms cause the production of toxins. Algal blooms may consist of

strains not actively producing toxic metabolites, or producing several simultaneously; consequently, the toxicity of a bloom is difficult to establish. In addition, the use of copper sulfate in reservoirs to treat cyanobacteria blooms causes the cells to lyse and potentially release toxins. This uncertainty necessitates active source water control and monitoring of water quality for cyanobacterial toxins.

[Reservoir Management Strategies for the Control and Degradation of Algal Toxins](#) (order #91199), co-sponsored by CRCWQT, investigated cyanobacterial toxin degradation in reservoirs by toxin-degrading organisms and developed reservoir management approaches for the control of toxin production using an ecological model. Reservoir hydrodynamics and growth of algal species were successfully simulated with the computer model. The timing and magnitude of blooms were similar for the field and the simulated data sets. The model was extended to include toxin production and degradation, which when applied to any reservoir would predict the risk of cyanobacterial toxins. The study also determined that utilities cannot rely on biodegradation to control the cyanobacterial toxins, microcystin, and cylindrospermopsin in drinking water reservoirs because toxins can be present in the water column without toxin-degrading bacteria being present. Cylindrospermopsin can persist for months in the water column, suggesting that biodegradation does not always occur. Additionally, screening with PCR for microcystin-degrading organisms revealed that these organisms were not always present in toxic blooms of *Microcystis aeruginosa*.

Water Utility Guidance

In the early 1990s, several different countries, including Canada, Australia, and the United Kingdom, moved forward to develop health guidance levels for microcystin in drinking water. In order to manage the potential hazard of cyanobacterial toxins, water suppliers needed knowledge not only of health risks, but also of the nature and causes of cyanobacterial blooms, the methods of monitoring and controlling toxins, the effectiveness of water treatment practices in removing toxins, and strategies for preventing and mitigating toxic bloom development.

The Foundation report, [Cyanobacterial \(Blue-Green Algal\) Toxins: A Resource Guide](#) (order #90693), published in 1995, provides utilities with a comprehensive guide to addressing algal toxin concerns, including strategies for communicating with the public on potential risks of these toxins. The guide outlines several tiered approaches to managing, monitoring, and analyzing toxins. As an example, the monitoring plan, originally developed in Australia, introduces a graduated response and action level to increasing levels of toxic cyanobacteria in source waters. In Australia, an Alert Levels Framework developed in the 1990s as a monitoring and management action sequence, provides a graduated response to the onset and progress of a cyanobacterial bloom. For instance, in the framework, Alert Level 1 (cyanobacterial biomass 2,000 cells/mL, 0.2 mm³/L biovolume, or 1 mg/L chlorophyll a) is the first step at which management action is taken.

Over the past 20 years, a number of organizations in several different countries have conducted research on managing cyanobacteria and the toxins they produce. The work has been published in various papers, reports, and books, and presently no single document exists that has consolidated this knowledge. The Global Water Research Coalition, of which The Foundation

is a founding member, addressed the need for a single, comprehensive resource for water suppliers worldwide with the project, [*Guidance Manual for the Management of Toxic Algae*](#) (order #3148). The manual includes perspectives from several countries on health effects, reservoir management, analytical methods, and treatment technologies to mitigate several species of algal toxins.

Foundation Reports Cited in this Article

- *Guidance Manual for the Management of Toxic Algae* (2009, order #3148)
- *Treatability of Algal Toxins Using Oxidation, Adsorption, and Membrane Technologies* (2010, order #2839)
- *Reservoir Management Strategies for the Control and Degradation of Algal Toxins* (2008, order #91199)
- *Determination and Significance of Emerging Algal Toxins* (2007, order #91170)
- *Development of Molecular Reporters for Monitoring Microcystis Activity and Toxicity* (2007, order #2818)
- *Early Detection of Cyanobacterial Toxins Using Genetic Methods* (2007, order #91198)
- *Removal of Algal Toxins from Drinking Water Using Ozone and GAC* (2002, order #90904)
- *Assessment of Blue-Green Algal Toxins in Raw and Finished Drinking Water* (2001, order #90815)
- *Cyanobacterial (Blue-Green Algal) Toxins: A Resource Guide* (1995, order #90693)