A Freshwater Algal Toxin Guidance Document for Public Health Laboratories

MAY 2017

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES™

Laboratory User Guide

The goal of this toolkit is to provide resources for environmental public health laboratories considering implementation of freshwater algal toxin testing. Although marine algal toxins are another public health concern, the focus of this resource is algal toxins in freshwater systems. As currently planned, this document will be updated on an as-needed basis given federal regulatory and advisory updates, testing method changes and other relevant developments, such as the Fourth Unregulated Contaminant Monitoring Rule (UCMR4).

Referral to commercial products or companies in this document does not constitute APHL endorsement.

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Cover photo: MODIS satellite image of Lake Erie, 10/9/11, showing algae bloom. Courtesy of NOAA CoastWatch.

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I. History and Background

Exposure to toxins produced by certain algal species are an ecological, public and animal health concern. Harmful algal blooms (HABs) are an increase in the mass and volume of algal cells that could occur seasonally in both freshwater and marine systems. Blooms typically occur during the warmer part of the year (spring to early autumn) and require the optimal mix of light, heat, organic matter, water flow and nutrients to develop. A key factor in bloom surge is nutrient loading, both nitrogen and phosphorus. These factors may be exacerbated by the effects of a changing climate, such as more extreme weather events and warmer water temperatures. Recently, we have seen an increase in frequency, species variability and duration of HAB events in freshwater, especially by cyanobacteria. Cyanobacteria, historically known as blue-green algae, is a bacteria capable of producing toxins, also known as cyanotoxins.

Cyanobacteria are found in fresh, estuarine and marine waters, and moist soils. They may form cell filaments or colonies and can be found in all layers of the water column. Colonies can have air "bladders" at their center, allowing them to float to the surface or subsist below the surface, depending on water conditions. Some cyanobacteria (e.g., *Planktothrix*) can be found in bottom sediments, but they can float to the surface when mobilized by storm events or other sediment disturbances. Other cyanobacteria blooms may remain dispersed through the water column (e.g., *Cylindrospermopsis sp.*) and cause water discoloration. Although the bloom itself can negatively affect the aquatic environment (e.g., oxygen depletion, physical interferences), it is the cyanotoxins produced by the cyanobacteria that comprise the bloom that are most threatening to human health.

Cyanotoxins are produced by a variety of Cyanobacteria genera, including *Microcystis*, *Anabaena*, *Cylindrospermopsis* and *Planktothrix,* among others. These and other genera can produce multiple types of cyanotoxins:

Algal Toxin	Examples of cyanobacteria capable of producing this algal toxin (not an inclusive list)
microcystins	*Microcystis spp.*, *Anabaena*, *Planktothrix spp*.
anatoxins	*Microcystis*, *Anabaena sp.*, *Cylindrospermopsis*, *Planktothrix*
cylindrospermopsin	*Anabaena spp.*, *Cylindrospermopsis*
saxitoxins	*Anabaena sp.*, *Planktothrix spp*.
nodularin	*Nodularia*

To date, the four most commonly found algal toxins are microcystins, anatoxins, cylindrospermopsin and saxitoxins. Microcystins have at least 100 known variants (congeners) and other cyanotoxins, such as anatoxins (e.g., anatoxin-a), have a smaller number of congeners. Microcystin-LR is the most studied single congener. Total microcystins is a measure of all microcystin congeners found in the sample. In most cases involving toxic effects, cyanotoxins are produced inside the cyanobacteria cell and are released upon cell death. However, species such as cylindrospermopsin can release significant amounts (up to 50%) of their toxins without cell breaking (lysis). Saxitoxins, although more commonly found in marine waters, have also been detected in freshwater systems.

II. Evolving Public Health Concerns

HABs are an environmental problem in every region of the US. Health effects from cyanotoxin exposure can be significant, as illustrated by CDC's summary document, *Algal Bloom-Associated Disease Outbreaks Among Users of Freshwater Lakes—United States, 2009–2010*. To better understand exposure patterns, CDC has developed the One Health Harmful Algal Bloom System (OHHABS), a voluntary reporting system that collects environmental data and information about individual human and animal cases of HAB-associated illnesses. Reporting guidance can be found on the CDC OHHABS website.

Cyanotoxins are currently divided into three main classes based upon their effects on humans or animals, including pets, livestock and wildlife. Neurotoxins (e.g., anatoxins, saxitoxins) affect the nervous system, hepatotoxins (e.g., microcystins, cylindrospermopsin, nodularins) affect the liver and dermatoxins affect the skin. Other possible health effects from lifetime exposure to toxins such as cancer are being researched. The primary exposure route is ingestion of contaminated water, but skin contact and inhalation may also occur through recreational activities. Another form of exposure was reported in 1996, when 116 patients at a renal dialysis clinic in Brazil were exposed intravenously during dialysis to microcystin-contaminated water from a local reservoir. Although the dialysis machines had filters, they were not designed to work with untreated water. Most of the exposed population developed acute liver failure and 76 died. More recently, cyanotoxins have been identified in drinking water supplies, such as 2014's contamination event in the city of Toledo, OH, where a three-day "Do Not Drink" advisory was issued.

It is unknown how many people become ill from HABs annually in the US because individual cases of illness have not been tracked nationally. Waterborne and foodborne disease outbreaks (defined as similar illness occurring in two or more people after exposure to the same water or food item) related to HABs may be reported by health departments to CDC's National Outbreak Reporting System (NORS); however, reporting is limited to outbreaks, so individual cases are not recorded in this system, in contrast to OHHABS. More information can be found on this topic in CDC's Morbidity and Mortality Weekly Report, Table 2 (Reported exposure, health effects and health-care use resulting from harmful algal bloom-associated waterborne disease outbreaks in the US, 2009-2010).

Exposure can occur when HABs are visible, or even after cyanobacteria have died, decayed and can no longer be seen in the water. The bloom can die off, but toxins released from the cyanobacteria cells into the water can remain at toxic concentrations. This can impact raw water at drinking water intakes, treatment plant filters and processed, treated or finished water.

To combat these threats, state and municipal environmental public health programs are developing strategies to monitor public drinking and recreational water supplies for blooms and cyanotoxins. Protecting people and animals during and after HAB events involves cyanobacterial identification and cyanotoxin measurement in the affected water body (e.g., ambient lakes or ponds, rivers, estuaries and bays). In addition to water, fish could also be affected by HABs. Although not completely understood, cyanotoxins could cause toxic effects on fish and bioaccumulate.

It is not yet known what triggers cyanobacterial cells to produce cyanotoxins. Consequently, confirmation of toxic conditions cannot be determined simply through water body observation and HAB cyanobacteria species identification. Conclusive evidence should include any or all of these three activities:

1. Observance of HAB physical characteristics
2. Identification and enumeration of cyanobacteria through microscopic and/or molecular methods
3. Biochemical and/or chemical analysis of water for cyanotoxins

When a HAB occurs, it can visually change the water to the color of pea soup. The bloom may represent floating blue-green paint that can change colors such as blue, green, brown, yellow, orange or red. The surface may have green clumps, foam, scum or mats or may not be visible on the water surface. When the HAB dies and decomposes, the odor nearby may smell of rotting plants.

While recreational water restrictions or closures due to bloom observance and/or toxin-producing cyanobacteria species identification is a risk-averse approach, the public health risk and local economic repercussions may be considerable. Yet when the water body is a drinking water supply source and water toxin levels are above the threshold, orders for consumption restriction and recreational water closures or no contact are recommended.

Federal, World Health Organization and State Cyanobacterial and Cyanotoxin Guidelines

To help address these challenges, the US Environmental Protection Agency (EPA), the World Health Organization (WHO) and several states have developed or are developing guidelines for issuing HAB-related criteria and advisories. Federal drinking water advisory guidelines and draft recreational water quality criteria have been developed along with the understanding of when and how HAB toxin exposure occurs. States have issued advisories based upon water body use, such as drinking water supply or recreation. Some state's health departments, for example Iowa, have adopted legislative code changes to include microcystin toxin exposures as a reportable poisoning. Clinical symptoms of exposure can include abdominal pain, headache, sore throat, vomiting, nausea, dry cough, diarrhea, blistering around the mouth, pneumonia, and liver and kidney damage. The reportable poisoning status requires health care providers and all laboratories to report suspected or confirmed exposures to microcystins.

Drinking Water

In June 2015, EPA issued non-regulatory 10-Day Drinking Water Health Advisories for two cyanotoxins, total microcystins and cylindrospermopsin:

EPA 10-Day Drinking Water Health Advisories

Cyanotoxin	Bottle-fed infants and preschool children	School-age children to adults
Total Microcystins	0.3 µg/L	1.6 µg/L
Cylindrospermopsin	0.7 µg/L	3.0 µg/L

These advisories were issued as guidance for public drinking water systems to establish concentrations for which no anticipated adverse health effects will occur over a 10-day exposure period. A lower concentration level is recommended for bottle-fed infants and young children (up to six years old) because, relative to their body weight, they consume more water. Health effects support documents were developed by EPA for microcystins, cylindrospermopsin and anatoxin-a. No health advisories were issued for anatoxin-a due to inadequate health effects data.

Many states are implementing raw and finished drinking water screening according to these advisories. Other states and municipalities are asked to voluntarily monitor for various cyanotoxins, such as total microcystins. To assess the treatment plant performance, paired analysis of raw (collected at the plant intake) and finished water samples collected at the entry point to the distribution system at the end of the plant's residence time is strongly recommended by EPA. Through the upcoming 2018-2020 EPA Unregulated Contaminant Monitoring Rule (UCMR4) program, EPA will collect data to determine how often ten cyanotoxins occur in various public water systems across the US. Over a consecutive four-month period, laboratories in the program will monitor for total microcystins, six microcystin congeners, cylindrospermopsins, anatoxin-a and nodularin. This data will inform whether these cyanotoxins should be regulated through the Safe Drinking Water Act.

Recreational Water

In December 2016, EPA developed draft Human Health Recreational Ambient Water Quality Criteria and/or Swimming Advisories for Microcystins and Cylindrospermopsins based upon the same peer-reviewed science as the drinking water health advisories. These criteria and advisories, if they are finalized, will provide recommended total microcystins and cylindrospermopsin concentration levels that should be protective of human health based upon skin exposure and accidental ingestion during primary recreational water activities (i.e., swimming or other water activities where a high degree of bodily contact with the water and immersion and ingestion are likely).

These criteria are proposed health-based guidance and therefore do not take into consideration the economic impacts or feasibility of meeting these recommended concentrations. States and authorized Tribes can use the values to determine whether a recreational water body should be open, closed, or remain open

with warnings, but even if the criteria are finalized in the current form, states will not be required to enforce them. Other criteria that are scientifically defensible and protective of the water body's designated use can also be applied. If the water body exceeds these criteria for more than 10% of days during a recreational season up to a calendar year, EPA proposes that these data may be used to indicate long-term impairment from multiple short-term blooms.

2016 EPA Draft Human Health Recreational Ambient Water Quality Criteria

Cyanotoxin	Recommended concentration to be protective of human health given a primary contact recreational exposure scenario
Total Microcystins	4 µg/L
Cylindrospermopsin	8 µg/L

In 2003, WHO established microcystins concentration ranges based upon the relative probability of low, moderate, high and very high acute health effects during recreational water exposure:

WHO 2003 Recreational Water Guidance/Action Level

Relative Probability of Acute Health Effects	Cyanobacteria Cell Density (cells/mL)	Estimated Microcystin Levels (µg/L)*
Low	<20,000	<10
Moderate	20,000-100,000	10-20
High	100,000-10,000,000	20-2,000
Very High	>10,000,000	>2,000

**Microcystin concentrations were derived from the cyanobacterial cell density levels.*

Other international recreational water guidelines for cyanotoxins and cyanobacteria are available in Appendix A (p. 111) of EPA's draft Human Health Recreational Ambient Water Quality Criteria and/or Swimming Advisories for Microcystins and Cylindrospermopsins.

State guidelines are available in Appendix B (p. 121) of this same document. The state guidelines are commonly based on WHO's tiered or modified guidelines for cell counts and toxin concentrations. These guidelines may provide recommended qualitative observations, cyanobacteria cell counts and/or cyanotoxin concentrations as thresholds for posting recreational water advisories or closures. Recreational advisory levels vary by state. Many issue advisories in a tiered approach, such as "avoid contact" or "all contact with water is restricted." If a bloom is detected, the need to escalate an advisory level is determined by considering factors such as changes in bloom size and bloom intensity.

III. Methods

Environmental public health laboratories use analytical methods to detect and count cyanobacteria and to measure cyanotoxin concentrations to inform public health advisories and warnings. To fully characterize the immediate threat of an observed HAB, microscopic identification and enumeration should be coordinated with a biochemical and/or chemical analysis of cyanotoxins. The methods described below can generally be applied to both drinking water and source waters, depending upon the desired sensitivity level.

Cyanobacteria detection and counting methods include:

1. Microscopic identification
2. Density enumeration of cyanobacteria colonies and cells

3. Molecular/biochemical analyses
    a. Quantitative polymerase chain reaction (qPCR)
    b. Experimental biochemical methods

Cyanotoxin analytical methods include:

1. Biochemical: enzyme-linked immunosorbent assay (ELISA)
    a. ELISA field kits (qualitative)
    b. ELISA laboratory kits (semi-quantitative)
2. Chemical: liquid chromatography tandem mass spectrometry (LC/MS/MS) (quantitative)
    a. EPA LC/MS/MS methods
    b. Other LC/MS/MS methods

Cyanotoxin analytical techniques or tools can be used to analyze a water sample or homogenate of lysed (ruptured) cells, range from simple (ELISA field screening kits) to complex (LC/MS/MS), and produce qualitative, semi-quantitative and quantitative results. These methods have been developed for cyanotoxins targeted in EPA advisories (total microcystins and cylindrospermopsin), but many can be used with microcystin congeners, anatoxin-a and nodularin. The 2015 EPA drinking water health advisories has limited the list of currently available test methods to those capable of measuring targeted cyanotoxins at or below the advisory concentration criteria. Protein Phosphatase Inhibition Assays (PPIA) have been reported in the literature as useful screens for veterinary investigations and have also been applied to water samples. Subsequent analysis using LC/MS/MS is required to identify and quantify the type of microcystin congeners. However, no commercial PPIA assays have been developed to quantify microcystin concentration in water at this time.

While researchers are developing new and improved cyanotoxin analysis methods, environmental and public health agencies must continue to rely on currently available methods to help determine when health advisories should be issued. Other laboratory method considerations include sample preparation, proficiency testing, methods limitations and reporting limits, and are described within this section.

Cyanobacteria Detection and Counting Methods

Microscopic Identification and Density Enumeration

Microscopic examination and taxonomy continues to be a simple means of identifying cyanobacteria in the freshwater bloom. Cyanobacterial identification keys (e.g., Phyco Key and New England "Dirty Dozen") based on the morphological appearance as seen under the microscope are improving and are available for public use. Microscopy is also used to determine the cyanobacterial cell and colony density by counting them in water samples. Cell and colony density is an indication of the potential cyanotoxin concentration in the water. The addition of digital photography capability allows laboratories to submit photomicrographs to experts to verify or confirm cyanobacteria identification.

Surrogates such as chlorophyll or phycocyanin can indicate if a bloom is present or about to form. These pigments can be measured using field kits or samples can be collected and measured in the laboratory using standardized tests. Direct measures of algal colony and cell enumeration or biomass though better determine the potential for or the extent of contamination.

The USGS Field and Laboratory Guide to Freshwater Cyanobacteria Harmful Algal Blooms for Native American and Alaska Native Communities can provide further information on this topic.

Molecular Analyses: qPCR

More advanced tools routinely used by molecular biology laboratories, such as qPCR, are being applied to identify and quantify cyanobacteria when blooms are observed in recreational and surface freshwater supplies. In 2016, Ohio EPA began piloting the use of qPCR to monitor the presence and density of cyanobacteria in state surface water supply sources. The advantage of this approach is its ability to identify

and quantify cyanobacteria by DNA and replication. It is more expensive than microscopic identification and enumeration, but many more samples may be analyzed in a three-hour period and identification is definitive. The disadvantage of both microscopic and qPCR methods is their inability to elucidate whether cyanobacteria are actively producing toxins or if toxins have been released into the water. Knowing the cyanotoxin concentration in recreational and public water supplies during or after a bloom is an important consideration when deciding to issue public health advisories. Using ELISA and/or LC/MS/MS techniques in combination with microscope identification and/or qPCR can help determine if the cyanobacteria are actively producing cyanotoxin.

Experimental Biochemical Methods

New methods continue to be developed that harness both microscopy and molecular biology. Although not widely used, one method reportedly quantifies toxic and toxin-producing cyanobacterial cells by microscopic recognition of individual genes' fluorescence in situ hybridization (RING-FISH). This method cannot quantify toxin concentrations, but determines whether the cyanobacteria are the toxin-producing types. It is designed to be used in combination with flow cytometry for a high throughput of laboratory analyses. In the meantime, current analytical methods are constantly being improved to achieve lower detection limits or more specific results for specific cyanotoxin types.

Cyanotoxin Determination Methods

Biochemical: Enzyme-linked Immunosorbent Assay (ELISA)

ELISA applications are available for field screening and laboratory analysis. Field screening methods can produce qualitative results (i.e., presence/absence above a given concentration). Laboratory methods can semi-quantitatively (i.e., produce a reliable estimated concentration) measure specific cyanotoxin concentrations (e.g., total microcystins, cylindrospermopsin, anatoxin-a), but not individual microcystin congeners. Some state programs require only total microcystins determination and reporting, while others call for both total microcystins and other cyanotoxins.

ELISA field methods (qualitative)

Field test kits provide rapid screening results for the presence of microcystins or cylindrospermopsin in fresh water. They are commercially available as test strips or test reaction tubes from Abraxis and Envirologix and are targeted to specific cyanotoxins using immunoassay and ELISA technology. Field kits are capable of determining the presence of the targeted toxin above a given concentration. Test strips using an immunoassay technique are dipped into the water sample and then the test strip indicator will express a positive or negative sample. Multi-tube kits employ ELISA to measure the toxin concentration by comparing the color change in the sample's reaction tube to the colors of the standard reaction tubes representing the toxin concentration range in water. Field kits are useful when deciding to issue preliminary warnings while waiting for the laboratory to receive and test samples quantitatively. These kits should not be used to support EPA drinking water health advisories. Public water systems that rely on surface water supplies should consider using a more sensitive method such as the semi-quantitative laboratory ELISA ADDA-specific test for total microcystins (described in the next section).

ELISA laboratory methods

Abraxis Inc., a leading manufacturer of cyanotoxin ELISA kits, also produces an automated microtiter plate format analyzer to detect total microcystins (not individual congeners), cylindrospermopsin, anatoxin-a, nodularin and saxitoxin. When microcystins or cylindrospermopsin are detected in drinking water, the toxin concentration determined by ELISA laboratory methods are not considered a confirmatory test by EPA because the cross reactivity or response varies between microcystins congeners and their concentrations. However, they are approved to establish that the total toxin concentration is below EPA health advisory levels and signal additional LC/MS/MS confirmatory testing. LC/MS/MS provides speciation information and is less prone to interference than ELISA.

ELISA kits sensitive to total microcystins use a variety of antibodies isolated against microcystin-LR and microcystin-RR congeners, as well as recombinant antibody fragments and antibodies against the

amino acid ADDA, which represent any remaining congeners. These express a color signal, which are evaluated for intensity using a microplate reader at 450 nm to provide an estimated total microcystins concentration (µg/L). It is an assumption that the kit will provide a similar response to all congeners, but since it is not based on direct equivalency it is considered a semi-quantitative method. These kits generally have quantitation ranges from a 0.2 limit of quantitation (LOQ) to an upper limit of 5 µg/L. When microcystin-LR was used for calibration to ensure cross-laboratory consistency, the laboratory ELISA kit method detection limit (MDL) ranged from 0.04 to 0.2 µg/L of total microcystins. Unfortunately, ADDA can also be present in samples as degradates and interfere with the sample analysis for microcystins. The range of sensitivities/response of ELISA for different variants versus the microcystin-LR and microcystin-RR, which are typically used to standardize the ELISA methods, is well documented. Guo et al., 2017 (Analysis of Microcystins in Drinking Water by ELISA and LC/MS/MS in Journal-AWWA) found substantial variability in ELISA using known spiked concentrations because of the lack of congener specific standards, as well as interference by ozonated degradates. They also found quantitative differences comparing ELISA and LC/MS/MS paired sample analyses.

EPA Method 546, Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay, uses this ELISA technique. The Ohio Environmental Protection Agency Laboratory expanded EPA Method 546 in their standard operating procedures (Total (Extracellular and Intracellular) Microcystins – ADDA by ELISA) to include additional quality-control and sample-handling advice. Questions about this method may be addressed by Vandana Deshmukh (614.644.4240, Vandana.Deshmukh@epa.ohio.gov) or Nik Dzamov (614.644.4068, Nik.Dzamov@epa.ohio.gov).

EPA LC/MS/MS Methods

In 2015, EPA released two cyanotoxin analytical chemistry methods, EPA Methods 544 and 545, using liquid chromatography and tandem mass spectrometry detection. The analytical methods have the option to measure dissolved (extracellular) cyanotoxins, or intracellular cyanotoxins after a lysing step that releases the toxins from the cyanobacterial cells. To determine water treatment plant effectiveness when cyanobacteria are present in source water, it is important to analyze source water both with and without cyanobacteria cell lysing. This identifies if cyanotoxins are already present prior to the filtration process, which will likely cause the release of cell toxins. When quantifying the total cyanotoxin concentration (intracellular and extracellular), sample preparation should include cell lysis. Analyzing and comparing the total toxin concentrations of raw (untreated) and finished water samples at the same time accurately determines the concentration of cyanotoxin before and after treatment.

EPA method 544, Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), was developed to quantify total microcystins and nodularins. It requires solid phase extraction sample preparation to achieve the sensitivity required to meet the total microcystins advisory limits. The method is designed to separately extract these toxins from intracellular and extracellular portions of the drinking water sample and combine the two extracts prior to analysis. Microcystins and nodularin calibration standards must be commercially available to run the method. Enzo Life Sciences, Inc., Abraxis, Inc. Caymen Chemicals, Beagle BioProducts, Inc., Sigma-Aldrich, Greenwater Laboratories and National Research Council Canada are potential providers of these standards. Deuterated Microcystin-LR surrogate can be purchased from Cambridge Isotope Laboratories, Inc.

EPA method 545, Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by LC/MS/MS, was developed for the direct injection (no solid phase extraction required) of drinking water samples into the LC/MS/MS specifically for separation and detection of cylindrospermopsin and anatoxin-a. Compared to microcystins and other cyanotoxins, these two toxins produce a very strong signal response from the detector and direct injection allows for rapid testing. This method can determine the total or extracellular concentrations of these toxins in water. When ELISA testing determines cyanotoxin concentrations to be at or above advisory levels, EPA guidance requires LC/MS/MS analysis to confirm their presence and concentration.

LC/MS/MS methods analyze drinking water samples for simultaneous analysis of multiple individual cyanotoxins, including microcystin congeners, cylindrospermopsin and anatoxin-a. These methods are limited by the availability of calibration standards specific to these toxins. Most of the uncommon microcystin congeners are not currently commercially available, making it impossible to rule out their presence.

Other LC/MS/MS Methods

The LC/MS/MS technique, Using the MMPB technique to confirm microcystins concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS), accounts for all microcystin congeners as total microcystins. The MMPB method incorporates a pre-analytical sample oxidation step that exposes and targets the ADDA portion that is present in all microcystin congeners. Potassium permanganate is used to oxidize all microcystins and expose the ADDA-moiety-producing MMPB (3-methoxy-2-methyl-4-phenylbutyric acid) molecule. The MMPB produced represents all microcystins and eliminates the need to test for specific congeners. The LC/MS/MS is calibrated using a certified microcystin reference standard (microcystin-LR) and 4-phenylbutyric acid as the internal standard with method detection limits of 0.05 µg/L for total microcystins. This method can detect total microcystins concentrations at or below the EPA health advisory levels as a single method for conformational analysis. However, questions remain as to whether this technique is appropriate for analyzing total microcystins in treated water, since some chlorination methods and pH may interfere with the recovery of quality control compounds.

Another technique, Rapid and Efficient Analysis of Microcystins, Nodularin, Cylindrospermopsin, and Anatoxin-a in Drinking Water by LC Tandem MS, simultaneously analyzes 11 cyanotoxins (eight microcystins, nodularin, anatoxin-a and cylindrospermopsin) in drinking water. Small sample volumes and a direct dilution procedure decreased sample processing time from hours to minutes when compared to traditional solid phase extraction procedures. The method demonstrated good sensitivity with limits of detection (<100 ng/L), precision (relative standard deviation <20%) and accuracy for individual microcystins. To quantify total microcystins, this method sums the individual microcystin concentrations. This method is sensitive enough to achieve the EPA advisory levels for recreational water, but may not reach the required sensitivity for drinking water.

Sample Preparation

To release intracellular toxins and measure total toxins, the ELISA and LC/MS/MS methods require lysing the cyanobacterial cell walls in the samples prior to analysis. The most common approach is to freeze and thaw the entire drinking water sample three times prior to analysis by these methods. EPA Method 544 requires filtering the drinking water sample through a 0.4 µm filter and then applying a freeze/thaw lysing technique to the membrane filter in methanol. Once the cells are lysed, the methanol extract is combined with the solid phase-extracted extracellular portion and analyzed by LC/MS/MS to determine the total toxin concentration.

Lysing is particularly important for raw water samples collected prior to any public water supply filtration process, since this is the only place where intact cells should be. For a well-designed, well-operated public water system, lysing would not be expected to have a significant impact on finished water samples since cyanobacteria cells should not be present in the filtered, finished water at significant levels. Some analysts confirm raw-water lysing effectiveness (or to judge the need for finished-water lysing) by microscopically examining for intact algal cells. For more information on this topic, please refer to the drinking water section in “Federal, World Health Organization and State Cyanobacterial and Cyanotoxin Guidelines” of this document.

Proficiency Testing

Proficiency test (PT) samples are important to demonstrate a laboratory’s capability. At least one company, Abraxis, Inc., has established an ELISA proficiency testing program for laboratories determining cyanotoxins in drinking and recreational waters. The Abraxis cyanotoxin PT samples may contain preservatives that make them incompatible for LC/MS/MS analysis, so laboratories should check with the supplier before ordering PT samples. To participate in the PT program, laboratories must apply for permission. The 2017 Abraxis

Cyanotoxin Proficiency Test Program part numbers, schedule and sign-up application forms are posted on their website.

Method Limitations

Toxin concentrations can vary widely, even within the same species in the same water body. This limits cell enumeration or biomass measurement tests beyond the determination of bloom presence/absence. Field tests with insufficient sensitivity to establish water safety may instead be useful for establishing bloom geographical extent, or estimating algal cell count and toxin concentration relationships.

Reporting Limits

Raw water intake direct toxin measurements should use methods that meet the local finished water health advisory level (e.g., 0.3 µg/L total microcystins in the US, or 1.5 µg/L as microcystin-LR in Canada). For greatest value to the public water system, the reporting limits should be well below the level of concern to allow for treatment and operational adjustments prior to reaching that level.

IV. Additional Information

Raw Water

Public water system source waters known to be algal bloom-prone should have an algal bloom management plan. Guidance for developing a management and/or response plan is available from a number of sources, including:

1. EPA. Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water, June 2015, Office of Water (4606M) EPA 815-R-15-010,
2. EPA. Cyanotoxin Management Plan Template and Example Plans, November 2016, Office of Water (4607M) EPA 810-B-16-006
3. EPA. HABs Guidelines and Recommendations.
4. WHO. Management of cyanobacteria in drinking-water supplies, 2015, WHO/FWC/WSH/15.0320:
5. Health Canada. Cyanobacterial Toxins — Microcystin-LR, July 2002 Guidelines for Canadian Drinking Water Quality: Supporting Documentation, Federal-Provincial-Territorial Committee on Drinking Water
6. American Water Works Association. Cyanotoxins Resource Community, Occurrence and State Approaches for Addressing Cyanotoxins in US Drinking Water, Journal - American Water Works Association February 2017, 109, 2, 40-47. Product Number: JAW_0084599
7. USGS. Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste-and-Odor Studies in Lakes and Reservoirs, Scientific Investigations Report 2008–5038, Jennifer L. Graham, Keith A. Loftin, Andrew C. Ziegler and Michael T. Meyer

Water suppliers should have a tiered response plan that initiates testing based on the bloom growth phase and its proximity to water intakes. Higher tier responses should include increased testing frequency as toxin concentrations at the water intake increase, direct toxin testing at finished water taps, and finally issuance of health advisories if toxin levels in the finished water warrant them.

Laboratories Capable of Providing Freshwater Cyanotoxin Laboratory Analysis

While public health and governmental laboratories decide whether or not to build the capability and capacity to determine cyanotoxins in water, they may be asked to provide assistance to identify analytical services for programs they serve. Resources for finding a government or commercial laboratory capable of providing freshwater cyanotoxin analysis can be found on the EPA CyanoHABs and New England Interstate Water Pollution Control Commission websites.

V. Active HAB Workgroups

1. Interagency Working Group on the Harmful Algal Bloom and Hypoxia Research and Control; for questions or feedback email IWG-HABHRCA@noaa.gov
2. Cyanobacteria Assessment Network Project
3. National Harmful Algal Bloom Committee
4. New England Interstate Water Pollution Control Commission
   a. NEIWPCC Cyanobacteria/Cyanotoxin Testing Services List
5. NOAA National Phytoplankton Monitoring Network
6. Gulf of Mexico Alliance Water Quality Priority Issue Team Harmful Algal Blooms Workgroup
7. Global Lake Ecological Observatory Network Harmful Algal Bloom Working Group
8. USGS Kansas Algal Toxin Research Team
9. Inland HAB Discussion Group
10. Great Lakes Collaboratory of the Great Lakes Commission; for questions email mel.adam@glc.org

VI. Other Resources

1. EPA CyanoHABs Website
2. WHO Guidelines for Safe Recreational Water Environments
3. WHO Toxic Cyanobacteria in Water Guide
4. CDC Cyanobacteria Harmful Algal Bloom Toolkit
5. Ohio EPA
6. Ohio Sea Grant
7. North American Lake Management Society Inland HAB Program (Cyanobacterial information clearinghouse which includes access to resources from all 50 states and human/animal health information).
8. Toxic Algae News Network
9. Wisconsin Climate and Health Program Harmful Algal Blooms Toolkit

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

This publication was developed under Assistance Agreement No. 83483301 awarded by the US Environmental Protection Agency (US EPA). It has not been formally reviewed by US EPA. The views expressed in this document are solely those of APHL and EPA does not endorse any products or commercial services mentioned in this publication. This publication was funded 100% with federal funds. $1,400,000 in federal funds supported this program.

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