# Cyanotoxins: A Guidance Document for Public Health Laboratories

NOVEMBER 2021

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

# LABORATORY USER GUIDE

The goal of this guidance document is to provide resources for environmental public health laboratories implementing cyanotoxin testing. The focus of this resource is cyanotoxins in freshwater systems, although marine cyanotoxins are a public health concern. 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.

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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# HISTORY AND BACKGROUND

Cyanobacteria—historically known as blue-green algae—are a phylum of prokaryotic microorganisms capable of producing toxins, known as cyanotoxins. Exposure to cyanotoxins is an ecological, public and animal health concern. Cyanobacterial harmful algal blooms (cyanoHABs) are an increase in the mass and volume of cyanobacterial 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. A key factor in bloom surge is nutrient loading, both nitrogen and phosphorus. These factors are exacerbated by the effects of a changing climate, such as more extreme weather events and warmer water temperatures. Recent literature continues to report an increase in frequency, species variability and duration of cyanoHAB events.

Cyanobacteria are found in fresh, estuarine and marine waters, and some terrestrial environments. They may form cell filaments or colonies and are found in all layers of the water column. Some cyanobacteria can have air "bladders," vacuoles or gas vesicles, resulting in adjusted buoyancy that causes colonies to float to the surface or within the water body, depending on water conditions.

Some benthic cyanobacteria can be found in bottom sediments or attached to rocks, but they can float to the surface when mobilized by storm events and other sediment disturbances. Other cyanobacterial blooms may remain dispersed through the water column (e.g., *Cylindrospermopsis*), or remain at an isotherm and cause water discoloration. Although the bloom itself can negatively affect the aquatic environment (e.g., oxygen depletion), cyanotoxins produced by the cyanobacteria that comprise the blooms are most threatening to human health.

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

**Table 1:** Cyanotoxins, their associated health effects and the cyanobacteria that can produce these cyanotoxins

| Cyanotoxin | Health Effects[1] | Examples of cyanobacteria capable of producing this toxin[1,2,3] |
|---|---|---|
| Microcystins | Carcinogen, hepatotoxin, protein phosphatase inhibition | *Anabaena, Microcystis, Dolichospermum, Planktothrix, Aphanocapsa, Hapalosiphon, Nostoc, Oscillatoria, Anabaenopsis, Arthrospira, Woronichinia, Fischerella, Gloeotrichia, Phormidium, Pseudoanabaena, Synechococcus, Geitlerinema, Microcoleus, Raphidiopsis, Scytonema, Tychonema* |
| Anatoxins | Respiratory paralysis | *Anabaena, Dolichospermum, Aphanizomenon, Arthrospira, Cylindrospermum, Microcycstis, Oscillatoria, Phormidium, Planktothrix, Raphidiopsis, Cuspidothrix, Tychonema, Woronichinia, Geitlerinema, Lyngbya, Microcoleus* |
| Cylindrospermopsin | Carcinogen, hepatotoxin, protein phosphatase inhibition | *Anabaena, Dolichospermum, Aphanizomenon, Raphidiopsis, Chrysosporum, Cylindrospermopsis, Umezakia, Lyngbya, Microseira, Oscillatoria* |
| Saxitoxins | Respiratory paralysis, death | *Anabaena, Dolichospermum, Aphanizomenon, Raphidiopsis, Cylindrospermopsis, Lyngbya, Planktothrix, Oscillatoria, Cuspidothrix, Microseira* |
| Nodularins | Carcinogen, hepatotoxin, protein phosphatase inhibition | *Nodularia, Nostoc* |

[1]Wehr JD, Sheath RG, Kociolek JP. Freshwater Algae of North America: Ecology and Classification, 2nd Ed., 2015. Academic Press, Cambridge, MA
[2]Westrick JA and Szlag D. A Cyanotoxin Primer for Drinking Water Professionals, Journal AWWA, 2018.110(8) E1-E16.https://awwa.onlinelibrary.wiley.com/doi/10.1002/awwa.1088
[3]Interstate Technology Regulatory Council. Strategies for Preventing and Managing Harmful Cyanobacterial Blooms (HCBs). https://itrcweb.org/teams/active/hcb

To date, the four most commonly found cyanotoxin classes are microcystins, anatoxins, cylindrospermopsins and saxitoxins. There are well over 100 known variants (congeners) of microcystin, and other cyanotoxins, such as anatoxins (e.g., anatoxin-a), have a smaller number of known 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, cyanotoxins are produced inside the cyanobacteria cell and are released upon cell death. However, species such as *Cylindrospermopsis* can release significant amounts (up to 50%) of their toxins without cell-breaking (lysis). Saxitoxins have been detected in freshwater systems, though they are most commonly found in marine waters produced by dinoflagellates of the genus *Alexandrium* and other genera.

## EVOLVING PUBLIC HEALTH CONCERNS

CyanoHABs are an environmental health problem in every region of the United States. Health effects from cyanotoxin exposure can be significant, as illustrated by the US Centers for Disease Control and Prevention's (CDC) summary document, Algal Bloom-Associated Disease Outbreaks Among Users of Freshwater Lakes—United States, 2009–2010. To 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 cyanoHAB-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 cyanotoxin exposure, 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. Many of the exposed individuals developed acute liver failure and 76 patients died. More recently, cyanotoxins have been identified in drinking water supplies, such as in 2014 and 2018 contamination events in the cities of Toledo, OH and Salem, OR, respectively. "Do Not Drink" advisories were issued in Toledo for the entire population for three days and in Salem for children and vulnerable populations for nearly four weeks.

It is unknown how many people become ill from cyanoHABs annually in the US. CyanoHAB-related waterborne and foodborne disease outbreaks (defined as similar illness occurring in two or more people after exposure to the same water or food item) may be reported by health departments to CDC's National Outbreak Reporting System (NORS). Since reporting is limited to outbreaks, individual cases are not recorded in this system, likely resulting in an underestimation of cases. 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).

To help remediate this gap in reporting, CDC established the OHHABS reporting framework by integrating the technical expertise gained from a 2007-2011 HAB surveillance project and NORS data. Eighteen states have adopted use of OHHABS, entering in 421 reports between 2016-2018, including information about 389 human illnesses and at least 413 cyanoHAB-associated animal illnesses. Additional information can be seen in the 2020 CDC report, *Surveillance for Harmful Algal Bloom Events and Associated Human and Animal Illnesses—One Health Harmful Algal Bloom System, United States, 2016-2018*.

Cyanotoxin exposure can occur when cyanoHAB blooms 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 (which may be distant from the bloom itself), treatment plant filters, and processed, treated or finished water. In recent years, there have been dog deaths associated with exposure to cyanotoxins in lakes and rivers.

To combat these threats, state and municipal environmental public health programs are developing strategies to monitor

public drinking water sources and recreational waters for blooms and cyanotoxins. Protecting people and animals during and after cyanoHAB events involves cyanobacterial enumeration and cyanotoxin measurement in the affected water body (e.g., lakes or ponds, rivers, estuaries and bays). Fish living in these water bodies can also be affected by cyanoHABs. Although not completely understood, cyanotoxins can bioaccumulate in their intestines, causing fish consumption advisories for impacted bodies of water.

It is unknown which factors drive the magnitude of cyanotoxin production by cyanobacterial cells. Consequently, confirmation of toxic conditions cannot be determined simply through water body observation and cyanobacteria species identification. Conclusive evidence should include observation and/or identification and enumeration, combined with biochemical and/or chemical analysis. For the following list, this would translate to #1 and/or #2, always in combination with #3:

1. Observance or history of water with physical or chemical characteristics consistent with cyanoHABs (e.g., color, blooms visibly present, bad odor due to production of geosmin and 2-Methylisoborneal (MIB) chemical compounds, elevated nitrogen or phosphorus levels, warm temperature and the presence of elevated phycocyanin levels (pigment-protein complex that biologically functions cooperatively with chlorophyll in cyanobacteria photosynthesis) in comparison to chlorophyll-a levels)
2. Identification and enumeration of cyanobacteria through microscopic and/or molecular methods
3. Biochemical and/or chemical analysis of water for cyanotoxins

When a bloom occurs, the water color can visually change to become discolored (murky) with a variety of colors including purple, pink, blue, brown, yellow, and/or orange, but are most frequently observed as green or red. The surface may have green clumps, foam, scum or mats, or may not even be visible on the water surface. When the bloom 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 are a risk-averse approach, the local economic repercussions may be considerable. However, when the water body is a drinking water supply source or recreational water body and the water cyanotoxin levels are above the US Environmental Protection Agency (US EPA) health guidance limits, consumption restriction orders from this source and recreational water closures or no-contact postings should be considered.

## Federal, World Health Organization and State Cyanobacterial and Cyanotoxin Guidelines

To help address these challenges, the US EPA, World Health Organization (WHO) and several states have developed or are developing guidelines for issuing cyanoHAB-related water quality criteria and advisories for drinking and recreational waters. Federal drinking water advisory guidelines and recreational water quality criteria have been developed along with the understanding of when and how cyanoHAB toxin exposure occurs. States have issued advisories based upon water body use, such as drinking water supply or recreation. Some state health departments, such as Iowa and Oregon, have adopted legislative code changes to include microcystin toxin exposures as a reportable poisoning. The reportable poisoning status requires health care providers and all laboratories to report suspected or confirmed exposures to microcystins to the health departments and/or regulatory agencies.

### Drinking Water

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

**Table 2:** US 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 |

https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins

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

Many states are implementing raw (collected at the plant intake) and finished (collected at the entry point to the distribution system) drinking water screening to these advisory levels. 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 and finished water samples synoptically (reflective of treatment process travel time) is strongly recommended by US EPA. During the 2018-2020 US EPA Unregulated Contaminant Monitoring Rule (UCMR4) program, US EPA collected data to determine how often four cyanotoxins, including six microcystin congeners, occur above the minimum reporting level (MRL) in various public water systems across the US. Over a consecutive four-month period, laboratories in the program monitored for total microcystins, six microcystin congeners, cylindrospermopsin, anatoxin-a and nodularin. These data will provide some of the necessary information to help US EPA determine whether these cyanotoxins should be regulated through the Safe Drinking Water Act (SDWA).

**Table 3:** UCMR4 Results Summary Data, April, 2021

| Cyanotoxin | Public Water Systems Tested (#) | Minimum Reporting Level (MRL) (µg/L) | MRL Exceedances (#) |
|---|---|---|---|
| Total Microcystins | 3,474 | 0.3 | 7 |
| –Microcystin-LA1 | 5 | 0.008 | 1 |
| –Microcystin-LF1 | 5 | 0.006 | 1 |
| –Microcystin-LR1 | 5 | 0.02 | 1 |
| –Microcystin-LY1 | 5 | 0.009 | 0 |
| –Microcystin-RR1 | 5 | 0.006 | 1 |
| –Microcystin-YR1 | 5 | 0.02 | 0 |
| –Nodularin-R1 | 5 | 0.005 | 0 |
| Anatoxin-a | 3,472 | 0.03 | 50 |
| Cylindrospermopsin | 3,472 | 0.09 | 12 |

1Samples for the microcystin congeners (e.g., microcystin-LA) and nodularin-R are only analyzed if the "total microcystins" result is ≥ 0.3 µg/L; thus, there are very few results for the individual congeners and nodularin-R.
MRL = UCMR Minimum Reporting Level. The minimum concentration that may be reported by a laboratory as a quantified value for a method analyte following analysis. The MRLs were established based on the capability of the analytical method, not based on a level established as "significant" or "harmful."
https://www.epa.gov/sites/production/files/2018-10/documents/ucmr4-data-summary.pdf

## Recreational Water

In May 2019, US EPA finalized Human Health Recreational Ambient Water Quality Criteria and/or Swimming Advisories for Microcystins and Cylindrospermopsin based upon the same peer-reviewed data as the drinking water health advisories. These criteria and advisories provide recommended total microcystins and cylindrospermopsin concentration levels that should be protective of human health based upon 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 health-based advisories 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 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, US EPA proposes these data may be used to indicate long-term impairment, as defined under the Clean Water Act.

**Table 4:** 2019 US EPA Human Health Recreational Ambient Water Quality Criteria[a]

| Application of Recommended Values | Microcystins | | | Cylindrospermopsins | | |
|---|---|---|---|---|---|---|
| | Magnitude (µg/L) | Duration | Frequency | Magnitude (µg/L) | Duration | Frequency |
| Recreational Water Quality Criteria | 8 | One in 10-day asessment period across recreational season | More than three excursions in a recreational season, not to be exceeded in more than one year[b] | 15 | One in 10-day assessment period across a recreational season | More than three excursions in a recreational season, not to be exceeded in more than one year[b] |
| Swimming Advisory | | One day | Not to be exceeded | | One day | Not to be exceeded |

[a] These recommendations can apply independently within an advisory program or in WQS. States can choose to apply either or both toxin recommendations when evaluating excursions within and across recreational seasons.
[b] An excursion is defined as a 10-day assessment period with any toxin concentration higher than the criteria magnitude. When more than three excursions occur within a recreational season and that pattern reoccurs in more than one year, it is an indication the water quality has been or is becoming degraded and is not supporting its recreational use. As a risk management decision, states should include in their WQS an upper-bound frequency stating the number of years that pattern can reoccur and still support its recreational use.
https://www.epa.gov/sites/default/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf page 76

In 2003, WHO established microcystin concentration ranges based upon the relative probability of low, moderate, high and very high acute health effects during recreational water exposure. In 2020, WHO issued additional guidance in *Toxic Cyanobacteria in Water, A Guide to Their Public Health Consequences, Monitoring and Management* (p. 300). Table 5 shows established Guideline Values (GV) based on ratios of microcystins to biovolume or chlorophyll-a concentrations that correspond to WHO health-based cyanotoxin exposure for drinking (chronic and acute) and recreational waters. These data were compiled from recent publications (reviewed in Section 2.1 and discussed in Section 4.6.2 of the 2020 WHO book) and establish alert levels (AL) that can be used to assume that if ALs are not exceeded, concentrations of microcystins are unlikely to exceed GV, thus allowing data-driven determinations of health-based risk.

**Table 5:** WHO 2020 Recreational Water Guidance/Action Level

| Alert Level | Biovolume MC/BV ≤ 3/1 [µg/mm³] | Chlorophyll-a MC/Chl.a ≤ 1:1 [µg/µg] | Basis for conservative estimate[a] of toxin/biomass |
|---|---|---|---|
| Alert Level 1 in drinking water ALF | 0.3 mm³/L | 1 µg/L | $GV_{chronic}$ for MCs in drinking-water: 1 µg/L |
| Alert Level 2 in drinking water ALF | 4 mm³/L | 12 µg/L | $GV_{short\text{-}term}$ for MCs in drinking-water: 12 µg/L |
| Alert Level 2 in recreational ALF | 8 mm³/L | 24 µg/L | $GV_{recreational}$ for MCs: 24 µg/L |

GV=Guideline Values
MC(s)=Microcystin(s)
ALF=Alert Level Framework
BV=Biovolume
Chl a=Chlorophyll-a

https://www.taylorfrancis.com/books/oa-edit/10.1201/9781003081449/toxic-cyanobacteria-water-ingrid-chorus-martin-welker
*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. 143) of US EPA's *Human Health Recreational Ambient Water Quality Criteria and/or Swimming Advisories for Microcystins and Cylindrospermopsin*.

State guidelines are available in Appendix B-2 (p. 156) of this same document. The state guidelines are commonly based on WHO's tiered or modified guidelines for cell counts and cyanotoxin 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 intensity.

## WORKING WITHIN THE ENVIRONMENTAL HEALTH SYSTEM

When a jurisdiction determines a cyanoHABs testing surveillance or emergency response program is needed to protect public health, appropriate stakeholders need to be aware of and included in the development of the program. Proactive, transparent and clear communication is essential to the development and implementation of a successful cyanoHABs testing program. A pre-testing summit with all stakeholders can help to set this precedent and develop consensus on the overall program goals, partner roles, information flow, and communication mechanisms. From there, regular follow-up meetings between appropriate stakeholders should be arranged to ensure the program is meeting established goals and proactive quality improvement and planning can take place.

Laboratories must be active contributors in this process by proposing clear procedures; proactively interfacing with system partners; establishing criteria that trigger sampling events; developing and communicating sampling, storage, and transport protocols with and for external partners; and reporting timely, accurate and clear results. Laboratories should work with partners to ensure understanding of the laboratory's capability, capacity, testing turnaround time and results reporting format (e.g., units: µg/L; document type: spreadsheet, PDF, hardcopy, electronic, etc.). If these parts of the process are clear, it is more likely the results will serve public health purposes and ensure public messaging is effective. The primary cyanoHAB system partners and their potential roles, particularly as they relate to the laboratory, are outlined in Table 6.

**Table 6:** Roles of CyanoHAB System Partners

| CyanoHAB System Partner | Role |
|---|---|
| Regulatory Agencies | Establishes cyanoHAB regulatory and health advisory standards. Laboratories will report results back to these agencies and should work with them to ensure testing capability and capacity to achieve these standards is attainable. |
| Waterbody Users (e.g., fishermen, boaters, swimmers) | May observe blooms and report them to governmental agencies. These groups will also be affected by closures due to blooms. |
| Water Utility and Reservoir Managers | Likely to be attuned to when and how source waters should be sampled. They may also be able to provide analytical services. |
| State and Local Public and Private Laboratories | May test drinking and recreational water samples to determine that regulatory and health advisory standards. |
| Health Department/Department of Environment | Incidents of cyanoHABs will be reported to these governmental agencies, who may then go out in the field to observe and sample reported blooms. |
| Local Community and Economy Representatives | Communicates how cyanoHAB events affect community operations and may be an effective liaison to help the community understand why cyanoHAB events are occurring. |
| Subject Matter Experts | May work with the laboratory to speciate blooms and provide current research. |

To streamline cyanotoxin reporting processes, it is possible that the same protocols and systems used for other emergency reporting (i.e., foodborne outbreaks or flu) can be leveraged for cyanotoxins. An interagency response coordination plan, such as the one My Water Quality California uses, may be helpful to create. The *EPA Incident Action Checklist—Harmful Algal Blooms* may be another helpful resource.

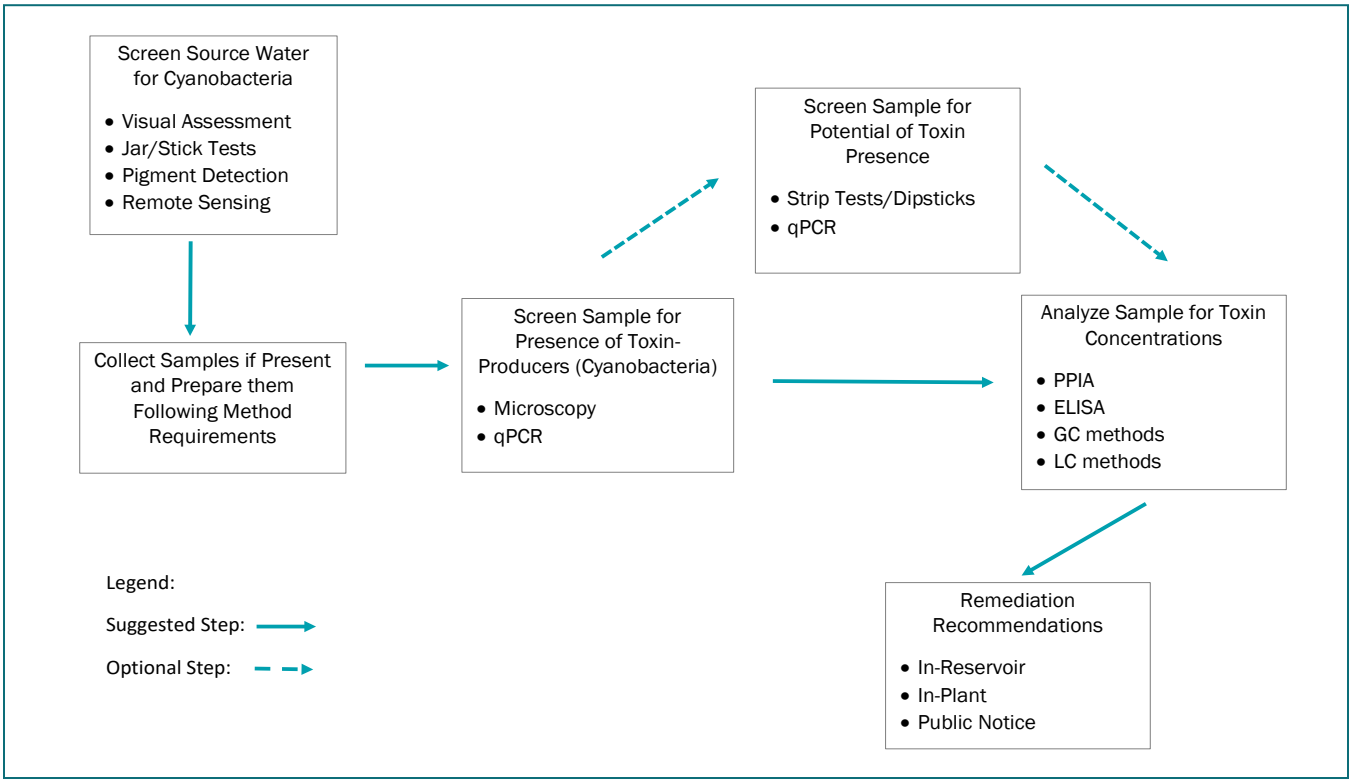
## Sampling

Laboratories or other cyanoHAB system partners might be responsible for sampling drinking or recreational water bodies for cyanotoxin analysis purposes. EPA's drinking and Oregon Health Authority's recreational water sampling guideline examples may be a useful reference for establishing proper protocols for your jurisdiction.

# 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 cyanoHAB, observation and microscopic identification/enumeration should be coordinated with a biochemical and/or chemical determination of cyanotoxins. The methods described below can generally be applied to drinking, source and recreational waters, depending upon the desired sensitivity level. They are the most common methods currently in use, but should not be viewed as an exhaustive list. The following flow chart outlines these methods and provides an example of a monitoring workflow.

**Figure 1:** Example CyanoHABS Monitoring Workflow




*All methods and their acronyms are described in subsequent text.

Cyanotoxin analytical techniques or tools can be used to analyze a water sample or homogenate-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 determination of the cyanotoxins targeted in US EPA advisories: total microcystins and cylindrospermopsin. The microcystin methods include nodularin and the cylindrospermopsin method includes anatoxins and saxitoxins. The 2015 US EPA drinking water health advisories have 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. However, few commercial PPIA assays are currently available to quantify microcystin concentration in water. Subsequent analysis using LC/MS/MS is required to identify and quantify the type of microcystin congeners, but this step is limited by the availability of certified reference standards.

While researchers are developing new and improved cyanotoxin analytical methods, environmental and public health agencies must continue to rely on current methods to make an informed decision on public health advisories. The specific methods, laboratory method considerations such as sample preparation, proficiency testing, methods limitations and reporting limits outlined in Figure 1 are discussed in more detail in the following sections..

## Screen Source Water for Cyanobacteria

### Visual Assessments

Visual assessment of water bodies is a qualitative method that is only suitable for cyanobacteria presence/absence determination. Many states provide guidance on recognizing cyanoHABs using visual cues. This detection method is easily employed by citizen science groups, which can help reservoir managers make early determinations on the need for further analysis. The Interstate Technology and Regulatory Council (ITRC) has developed a visual assessment guidance document in Appendix A of its website *Strategies for Preventing and Managing Harmful Cyanobacterial Blooms (HCBs)*.

### Jar and Stick Tests

Jar and stick tests are also qualitative methods only suitable for cyanobacteria presence/absence determination. A clear jar is used to collect a water sample and allowed to sit for 15-30 minutes. Cyanobacteria will float to the surface and algae and other sediment will settle out. Stick tests can be used to determine if a bloom consists of planktonic cyanobacteria or green algae. A stick is dipped into the bloom-affected area. Cyanobacteria will coat the stick, while green algae rarely do. These are quick field tests that can be done with little expertise and can also be employed by citizen science groups.

### Pigments

Pigment methods are quantitative and can detect cyanobacteria presence/absence and density. Algae produce chlorophyll-a, while cyanobacteria produce chlorophyll-a and phycocyanin. Both are pigments. Pigment detections can be done in the field (fluorometric probes) or in the lab (fluorometric probes, spectrophotometry or spectrometry). Ratios of chlorophyll-a to phycocyanin can be used to determine density estimates of green algae to cyanobacteria (e.g., when phycocyanin concentrations are low, cyanobacteria concentrations are low). This can be used to determine if blooms are likely to be toxic due to cyanobacteria presence, or non-toxic if they mostly consist of non-toxic green algae. It does not reflect cell counts.

### Remote Sensing

Remote sensing is a method that can be qualitative or quantitative and can be used to detect cyanobacteria presence/absence and density. It is employed by satellites and airborne systems, e.g., drones, and measures light energy reflected from the water surface. A combination of spectral channels, primarily in the red and near infrared channels for cyanobacteria, can be used to generate quantitative data on cyanobacteria biomass and other water quality changes.

## Screen Sample for Presence of Toxin-Producers (Cyanobacteria)

### Microscopic Identification and Density Enumeration

Microscopic examination and taxonomy continues to be a technologically simple means of identifying cyanobacteria in the freshwater bloom, but requires some level of taxonomical expertise. Cyanobacterial identification keys (e.g., Phyco Key, New England "Dirty Dozen," FlowCam Image Guide, ITRC Visual Guide) 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 are indications of the potential cyanotoxin concentration in the water. The addition of digital photography capability allows laboratories to submit photos to experts to verify or confirm cyanobacteria identification.

Flow-imaging microscopy is also a useful tool. This technology allows a greater number of samples to be processed but lacks the resolution of traditional light microscopy. Greater sample volumes can be analyzed quickly for the presence of cyanobacteria, but often only to the functional group or genus-level. This is important to note because not all species of a cyanotoxin-producing genus are always cyanotoxin producers (e.g., many species of *Microcystis* produce microcystin, but not all.) The presence alone does not indicate cyanotoxin production. While nothing completely replaces traditional microscopic identification methods, this is a highly effective tool when its limitations are understood. Surrogates such as chlorophyll or phycocyanin can indicate if a bloom is present or about to form. These pigments can be measured in situ with data logging sondes, using field kits, or samples can be collected and measured in the laboratory using standardized tests. Direct measures though of algal colony and cell enumeration or biovolume can 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* and the *ITRC Strategies for Preventing and Managing Harmful Cyanobacterial Blooms (HCBs)* can provide further information on this topic.

### Molecular Analyses: Quantitative Polymerase Chain Reaction (qPCR) and Digital PCR

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, the Ohio EPA began piloting the use of qPCR to monitor the presence and density of cyanobacteria and the presence and number of cyanotoxin-producing genes found in surface water supply sources. California EPA's State Water Resources Control Board also began piloting the use of qPCR in 2017 to monitor the presence of cyanotoxin-producing blooms in state recreational water bodies. The advantage of this approach is its ability to quantify the relative abundance of cyanobacterial and cyanotoxin-producing genes by using molecular-based assays to detect the presence of target genes.

qPCR is more expensive than microscopic identification and enumeration, but many more samples may be analyzed in a three-hour period and gene detection is more conclusive when determining the likelihood of cyanotoxin presence. For example, the presence alone of *Microcystis* determined by microscopy is not indicative of cyanotoxin presence because several species produce this toxin. But the presence of the microcystin-producing gene mcyE is conclusive in confirming the potential for microcystin production because the genetic blueprint for toxin production is present. One commercially-available kit from Phytoxigene allows for the detection and quantification of cyanotoxin-producing genes.

Another form of PCR gaining popularity that can be utilized for this type of molecular analysis is digital PCR (dPCR). dPCR uses Poisson statistics to calculate an absolute measurement of genetic material by partitioning a sample into thousands of reactions and counting positive reactions. It may be preferred when enhanced performance over qPCR is needed if the sample contains additional environmental and/or sample prep constituents that can inhibit the production of an accurate qPCR standard curve. BioRad's droplet digital PCR (ddPCR) is a type of dPCR in which samples are partitioned into 20,000 individual droplets and amplification occurs in each individual droplet, effectively eliminating inhibition issues. In a qPCR-ddPCR comparison study using *Microcystis* and *Cylindrospermopsis*, qPCR had higher sensitivity, a wider linear dynamic range, a shorter analysis time, and was more cost-effective. However, ddPCR has lower variability and inhibition was reduced, which achieved a higher degree of precision and accuracy.

The disadvantage of both microscopic and molecular methods is their inability to indicate whether cyanobacteria are actively producing cyanotoxins or if cyanotoxins have been released into the water above analytical detection limits. 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 microscopic identification and/or qPCR in combination with ELISA and/or LC/MS/MS techniques can help determine cyanotoxin presence and concentration. These techniques are discussed later in this document.

### 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 gene fluorescence in situ hybridization (RING-FISH). This method cannot quantify cyanotoxin 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.

## Screen Sample for Potential of Toxin Presence

### Strip Tests/Dipsticks and qPCR

Strip tests and dipsticks allow a quick determination of cyanotoxins presence. They are semi-quantitative and can be used to determine cyanotoxin presence/absence and a relative concentration. Visual assessments cannot confirm cyanotoxins, but strip tests and dipsticks can be used in the field with visual assessments to determine if further testing is needed. In this type of test, an antibody/antigen reaction is used to drive a color change on a strip. Color change intensity indicates relative cyanotoxin concentration and can be used to determine subsequent analytical steps. The qPCR methods mentioned above may also be used to screen for the presence of cyanobacteria.

## Sample Preparation

When preparing the sample for analysis, the primary consideration is to decide whether the sample should be analyzed as is to determine extracellular cyanotoxin concentrations (filtered or non-filtered depending upon water quality), or if it should be lysed to also determine intracellular cyanotoxin concentrations. To release intracellular toxins and measure total toxins, the ELISA and LC/MS/MS methods mentioned in the following section require lysing the cyanobacterial cell walls in the samples prior to analysis. The most common lysis approach is to freeze and thaw the entire drinking water sample three times prior to analysis. US 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. US EPA methods 544 and 546 use amber glass sample containers to prevent loss of microcystins by absorption to plastic materials. Lysing is particularly important for raw water samples collected prior to any public water supply filtration process, as cyanotoxins may enter a treatment plant either as extracellular (outside the cell in the water column) or intracellular (within the cyanobacterial cell with the possibility of release within the treatment plant). 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.

## Analyze Samples for Toxin Concentration

### Protein Phosphatase Inhibition Assay (PPIA)

PPIAs are quantitative tests for microcystin and nodularins and can be used to determine cyanotoxin presence/absence and concentration. It is a colorimetric assay that relies on enzyme inhibition, a biological effect of cyanotoxins. This is unlike ELISA or mass spectrometry, which use cyanotoxin molecular structure to determine concentration.

### 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, saxitoxin). Some state programs require only total microcystins (as can be measured by EPA 546) determination and reporting, while others call for both total microcystins and other cyanotoxins (measured by LC-MS/MS).

*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 Eurofins Abraxis and Envirologix and are targeted to specific cyanotoxins using ELISA technology. Field kits are capable of determining the presence of the targeted cyanotoxin above a given concentration. Test strips are dipped into the water sample and then the test strip indicator will express samples as positive or negative based on an immunoassay reaction. Multi-tube kits employ ELISA to measure the cyanotoxin concentration by comparing the color change in the sample's reaction tube to the colors of the standard reaction tubes representing the cyanotoxin 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. If a cell lysis step (as per the kit instructions) is not a part of the field method, then the result can underestimate the cyanotoxin concentration. These kits should not be used to support US 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 (semi-quantitative)*

Eurofins Abraxis, Inc. also produces an automated microtiter plate format analyzer to detect and quantify total microcystins (not individual congeners/nodularins), cylindrospermopsin, anatoxin-a, and saxitoxin. The use of these technologies in ELISA laboratory methods can establish whether the total toxin concentration is below US EPA health advisory levels.

ELISA kits sensitive to total microcystins use a variety of antibodies isolated against microcystin-LR and microcystin-RR congeners. They also use recombinant antibody fragments and antibodies against the amino acid ADDA, which is present in most of the 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 in µg/L. The kit assumes a similar response is provided for 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 µg/L 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 will quantify as microcystins. The range of sensitivities and 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. (*Analysis of Microcystins in Drinking Water by ELISA and LC/MS/MS*) 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, so interlab studies should specify the method for quantitative comparison.

US 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 EPA Laboratory expanded US EPA Method 546 in their standard operating procedures (Total (Extracellular and Intracellular) Microcystins – ADDA by ELISA) to include additional quality-control and sample-handling criteria. 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).

## US EPA LC/MS/MS Methods

To measure microcystins, targeted LC/MS/MS analysis may be used to provide specific congener concentrations. LC/MS/MS is generally more sensitive and less prone to interference than ELISA. However, LC/MS/MS should not be used as microcystin confirmation of ELISA as the cross reactivity or response with ELISA varies between microcystins congeners and their concentrations. Targeted LC/MS/MS only measures for the microcystin congeners with commercially-available standards for that method and not the total microcystins concentration. US EPA has not evaluated non-targeted LC/MS/MS analytical methods for the determination of cyanotoxins in drinking water, nor comparisons between ELISA and LC/MS/MS for other individual cyanotoxins, e.g., cylindrospermopsin and anatoxin-a.

In 2015, US EPA released two drinking water cyanotoxin analytical chemistry methods, US EPA Methods 544 and 545, using liquid chromatography and tandem mass spectrometry detection. In 2017, US EPA released two single laboratory-validated ambient water LC-MS/MS methods. 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 intracellular 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 synoptically accurately determines the concentration of cyanotoxin before and after treatment.

US 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 selected microcystins and nodularins. It requires solid phase extraction sample preparation to achieve the sensitivity required to meet the microcystins advisory limits for six microcystin congeners. The method is designed to separately extract these cyanotoxins from intracellular and extracellular portions of the drinking water sample and combine the two extracts prior to analysis. Microcystins and nodularin calibration standards are commercially available to run the method from the following vendors: Enzo Life Sciences, Inc., Eurofins Abraxis, Inc., Caymen Chemicals, Beagle BioProducts, Inc., Sigma-Aldrich, Greenwater Laboratories and National Research Council Canada. Other vendors may also offer these standards. Deuterated Microcystin-LR surrogate can be purchased from Cambridge Isotope Laboratories, Inc.

US EPA method 545, *Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-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. By splitting samples and doing a filtered and unfiltered analysis, this method can determine the total or extracellular concentrations of these cyanotoxins in water.

## Other Methods

Another LC/MS/MS approach that uses an MMPB (3-methoxy-2-methyl-4-phenylbutyric acid) molecule technique to confirm microcystins concentrations in water measured by ELISA and high-performance liquid chromatography (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 microcystin congeners. Potassium permanganate is used to oxidize all microcystins and expose the ADDA-moiety-producing MMPB 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 US 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 target analytes and quality control compounds.

Another technique, *Rapid and Efficient Analysis of Microcystins, Nodularin, Cylindrospermopsin, and Anatoxin-a in Drinking Water by LC Tandem MS*, simultaneously determines 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 US EPA advisory levels for recreational water but may not reach the required sensitivity for drinking water. For a summary of the US EPA cyanotoxin methods, see Table 1 in Appendix A.

## Proficiency Testing

Proficiency test (PT) samples are important to demonstrate a laboratory's capability. At least one company, Eurofins Abraxis, Inc., has established a PT program for laboratories determining cyanotoxins in drinking and recreational waters. Some 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. The current Eurofins Abraxis Cyanotoxin PT Program part numbers, schedule and sign-up application forms are posted on their website.

## Method Limitations

Cyanotoxin concentrations can vary widely, even within the same species in the same water body. This limits the accuracy of 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 cell count and cyanotoxin concentration relationships.

## Reporting Limits

Raw water intake cyanotoxin 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.

# REPORTING RESULTS

After analysis, the laboratory reports the results to the submitter and/or agency(ies) identified during the planning process, such as the state health department or department of environmental management/quality. The laboratory's Laboratory Information Management System (LIMS) can be used for this purpose and can be set up to send reports directly to these agencies, whose communications departments will take the results from the laboratory to create public messaging based on EPA advisories and/or their jurisdictional standards. Both cyanotoxin concentrations and cyanobacteria counts should be reported in the agreed-upon units and format (e.g., units: µg/L; document type: spreadsheet, PDF, hardcopy, electronic, etc.) to accurately identify the type and quantity of toxin in the water. Results may be reported for drinking and/or recreational water bodies and may need to be shared across multiple municipalities or states. The results may then uploaded to a state database such as Oregon's Water Quality Monitoring System or a national database such as OHHABs.

Helpful resources for reporting results include:

- Interstate Technology Regulatory Council: *Strategies for Communication and Response Planning for HCBs*
- *Communicating about Cyanobacterial Blooms and Toxins in Recreational Waters*
- *ORSANCO Harmful Algae Bloom Monitoring, Response and Communication Plan*

# PUBLIC MESSAGING

Health departments, departments of environmental management/quality and some laboratories have public information officers (PIO) who craft clear, understandable messages that may include laboratory results. Laboratories work with the PIOs to ensure laboratory result uses and limitations are understood. Advisories may be posted to notify boaters, fishermen, swimmers and the general public of water bodies with potentially harmful cyanotoxin levels. Advisories should remain posted until follow-up testing determine the area is below health advisory levels. Public messaging topics may include waterbody closures and advisories, actions to protect human and animal health, exposure symptoms, treatment options, real-time resources and contact information. Examples of public messaging may be found below:

- Oregon Health Authority: Cyanobacteria Blooms
- EPA Drinking Water Cyanotoxin Risk Communication Toolbox
- My Water Quality California: HAB Outreach and Communication

- CDC Health Promotion Materials
- EPA Templates and Generic Examples of Public Messaging

# ADDITIONAL INFORMATION

## Raw Water

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

1. US EPA. *Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water*, June 2015, Office of Water (4606M) EPA 815-R-15-010,
2. US EPA. *Cyanotoxin Management Plan Template and Example Plans*, November 2016, Office of Water (4607M) EPA 810-B-16-006
3. WHO. *Management of cyanobacteria in drinking-water supplies*, 2015, WHO/FWC/WSH/15.0320:
4. Health Canada. *Cyanobacterial Toxins – Microcystin L-R July 2002 Guidelines for Canadian Drinking Water Quality: Supporting Documentation*, Federal-Provincial-Territorial Committee on Drinking Water
5. 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
6. 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
7. US EPA. *Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin*.
8. US EPA. *Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems*.
9. US EPA. *EPA Tools for Waterbody Managers to Monitor for and Respond to CyanoHABs*.

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 cyanotoxin concentrations at the water intake increase, direct cyanotoxin testing at finished water taps, and issuance of health advisories if cyanotoxin levels in the finished water warrant them.

### Laboratories Capable of Providing Cyanotoxin Laboratory Analysis

While public health and governmental laboratories decide whether 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 cyanotoxin analysis can be found on the US EPA CyanoHABs and New England Interstate Water Pollution Control Commission websites.

# ACTIVE HAB PROJECTS AND 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. Great Lakes HABs Collaborative (email kgibbons@glc.org with questions)

## OTHER RESOURCES

1. US 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. Wisconsin Climate and Health Program Harmful Algal Blooms Toolkit
9. ITRC Strategies for Preventing and Managing Harmful Cyanobacterial Blooms (HCBs)
10. US EPA State HABs Resources
11. US EPA Cyanotoxins Preparedness and Response Toolkit

# APPENDIX A: SUMMARY OF CYANOTOXIN DETECTION METHODS FROM US EPA

| Cyanotoxins | | | | |
|---|---|---|---|---|
| **Methods** | **Anatoxins** | **Cylindrospermopsins** | **Microcystins** | **Saxitoxins** |
| **Biological Assays** | | | | |
| Mouse | Yes | Yes | Yes | |
| Protein Phosphatase Inhibition Assays (PPIA) | No | No | Yes | |
| Neurochemical | Yes | No | No | |
| Enzyme-Linked Immunosorbent Assays (ELISA) | Yes | Yes | Yes | Yes |
| **Chromatographic Methods** | | | | |
| ***Gas Chromatography*** | | | | |
| Gas Chromatography with Flame Ionization Detection (GC/FID) | Yes | No | No | No |
| Gas Chromatography with Mass Spectrometry (GC/MS) | Yes | No | No | No |
| ***Liquid Chromatography*** | | | | |
| Liquid Chromatography / Ultraviolet- Visible Detection (LC/UV or LC/PDA) | Yes | Yes | Yes | Yes |
| Liquid Chromatography/Fluorescence (LC/FL) | Yes | No | No | Yes |
| ***Liquid Chromatography Combined with Mass Spectrometry*** | | | | |
| Liquid Chromatography Ion Trap Mass | Yes | Yes | Yes | Yes |
| Liquid Chromatography Time-of-Flight Mass Spectrometry (LC/TOF MS) | Yes | Yes | Yes | Yes |
| Liquid Chromatography Single Quadrupole Mass Spectrometry (LC/MS) | Yes | Yes | Yes | Yes |
| Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/MS/MS) | Yes | Yes | Yes | Yes |

https://www.epa.gov/ground-water-and-drinking-water/detection-methods-cyanotoxins

## 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 project was 100% funded with federal funds from a federal program of $80,000. This publication was supported by Cooperative Agreement #NU60OE000104 from the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or the Department of Health and Human Services.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