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Recommendations for Cyanobacteria and Cyanotoxin Monitoring in Recreational Waters

Foreword

The purpose of this document is to provide EPA's recommendations, information and process steps that a recreational water manager or public health official may choose to follow, or adapt, to aid in determining if there is a harmful bloom or cyanotoxins posing a risk to humans, pets, wildlife and livestock in a water body.

This document provides EPA's monitoring recommendations; nonetheless, EPA has provided general information on these issues on the cyanotoxin webpage, <u>Monitoring and Responding to</u> <u>Cyanobacteria and Cyanotoxins in Recreational Waters</u>. For a stepwise conceptual cyanotoxin monitoring program framework:

- Step 1: Assess vulnerability of the water body to HABs and prioritize recreational waters for monitoring;
- Step 2: Observe recreational water body for blooms at the beginning and throughout the recreational season;
- Step 3: Monitor for cyanotoxins; and,
- Step 4: Follow up cyanotoxin monitoring.

Disclaimer

This document provides the EPA's recommendations for cyanobacteria and cyanotoxin monitoring in recreational waters. These monitoring recommendations do not impose legally binding requirements on the U.S. Environmental Protection Agency (EPA), states, tribes, or the public. These recommendations also do not confer legal rights. These recommendations do not constitute a regulation, nor do they change or substitute for any Clean Water Act (CWA) provision or the EPA regulations.

These recommendations may not apply to a particular situation based upon the circumstances. Interested parties are free to raise questions about the substance of these recommendations and the appropriateness of their application to a particular situation. The EPA retains the discretion to recommend approaches on a case-by-case basis that differ from those described in this document where appropriate. The EPA may revise this document periodically without public notice. The EPA welcomes public input on these recommendations at any time.

How to Monitor Cyanobacteria/Toxins in Recreational Waters*



*Adapted from "Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water" June 2015, EPA 815-R-15-010 ** This can either be an advisory/warning or a closure.

***If yes, consider modifying notification to indicate dangerous toxin level or cell count. If toxins are present but less than appropriate trigger value, continue to monitor toxins.

****If the state does not have a HAB program with a value for cyanotoxins or cell counts upon which to base a notification, recreational water managers may want to consider using the draft values that EPA recommends.

Recommendations for Cyanobacteria and Cyanotoxin Monitoring in Recreational Waters

Step 1: Assess vulnerability of the water body to cyanobacterial blooms and prioritize recreational waters for monitoring

Protecting public health is the primary objective for a monitoring program. To meet this objective, recreational water program managers and public health officials should make every effort to sufficiently characterize the water body to better understand the potential for harmful blooms, and thus the adverse public health risk that might occur in these waters. Sometimes phytoplankton (which includes cyanobacteria, microalgae, dinoflagellates and other microorganisms) can grow to high cell densities and form blooms. These blooms may or may not be toxic. This document focuses on cyanobacterial blooms with the potential for harmful cyanotoxins (also known as harmful algal blooms or HABs). A bloom can have extremely high cell densities of cyanobacteria (extremely high densities are typically defined as greater than 20,000 to 100,000 cells per mL) (Loftin et al., 2008). Cyanotoxins are produced by some toxic-producing species and are not always released into the water. Harmful algal blooms could adversely affect people and animals, regardless of the presence of toxins. Exposure to elevated cyanobacterial cells densities has been associated to dermal effects such as skin rashes, ear and eye infections and gastrointestinal distress. Harmful blooms are those that pose a health risk to people, either due to the presence of toxins or due to elevated densities.

1.1 Assess vulnerability of the water body to cyanobacterial blooms

Some recreational waters are more vulnerable than others based on the water body and water shed characteristics. Fast flowing, nutrient-poor rivers are less vulnerable than nutrient-rich lakes and reservoirs. Existing water quality data can help to determine if the water body has had a history of blooms or bloom indicators such as high cyanobacterial cell counts or chlorophyll-a levels. Elevated nitrogen and phosphorus levels will be important to consider in a waterbody evaluation. Waterbody assessments should consider the predominant land use in the watershed and potential nutrient sources that may lead to cyanobacterial growth for a system-specific evaluation. Similarly, climate and weather information such as water temperature and intensity of precipitation events will help to determine if conditions are conducive to increased levels of site-specific cyanobacterial growth currently and in the future.

A variety of information can be considered to assess the vulnerability of the recreational water to cyanobacterial blooms including: the type of water body; historical cyanotoxin occurrence; weather data (increases in temperature, precipitation and light); seasonal patterns of cyanobacterial blooms; land use patterns; physical and hydrologic factors (e.g., turbidity, pH and nutrients, and residence time); chlorophyll-a and phycocyanin levels; point and nonpoint sources of contamination upstream; water quality impairments; and, any other information gathered as part of source water assessments or sanitary surveys.

EPA recommends that managers, public health officials, tribes and states evaluate available data on their recreational waters to assess waterbody vulnerability to cyanobacterial blooms. Specifically, available data can be evaluated against EPA's recommended values or their own state/s values to determine whether harmful blooms have occurred in the past. As a benchmark for evaluating the risk to public health in recreational waterbodies, EPA has released a draft document for public comment that provides cyanotoxin values (<u>Recreational AWQC/Swimming Advisory for Cyanotoxins</u>). See Table 1.

Table 1. Draft EPA Recommended Values for Recreational Criteria and Swimming Advisories for Cyanotoxins

Microcystins	Cylindrospermopsin			
4 μg/L ^{a,b}	8 μg/L ^{a,b}			
a) Swimming Advisory: not to be exceeded on any day				

b) Recreational Criteria for Waterbody Impairment: not exceeded more than 10 percent of days per recreational season up to one calendar year.

Although EPA has recommendations for specific toxins, cell counts and/or biomass, together with microscopic identification can be informative and an interim step to make public health decisions and/or prompt toxin analysis. The Global Water Research Coalition, a non-profit organization for water research, published voluntary guidelines in 2009. <u>The International Guidance Manual for the Management of Toxic Cyanobacteria</u> provides information on many topics including cell enumeration, and calculation of biovolume/biomass. The World Health Organization (WHO) established guidelines for cyanobacterial cells (see Table 1) and several states (e.g., Connecticut, Indiana, Kentucky, Oklahoma, Utah, Wisconsin) use them for their swimming advisory level.

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Chlorophyll <i>a</i> (µg/L)	Estimated Microcystin Levels (µg/L) ^a
Low	< 20,000	< 10	< 10
Moderate	20,000–100,000	10–50	10–20
High	>100,000-10,000,000	50–5,000	20–2,000
Very High	> 10,000,000	> 5,000	> 2,000

Table 2. WHO (2003) Recreational Guidance/Action Levels for Cyanobacteria, Chlorophyll a, and Microcystin

^aWHO (2003) derived the microcystin concentrations from the cyanobacterial cell density levels.

1.2 Prioritize recreational waters for monitoring. Recreational water managers, public health officials or state water quality staff should develop a risk-based monitoring plan for recreational waters that are potentially vulnerable to blooms in order to prioritize their monitoring resources by considering the following information:

- Existing and historical recreational water quality,
- Sampling considerations,
- Analytical methods,
- Sampling/testing logistical considerations,
- Use of predictive tools and satellite data (see Section 2.2), and
- Frequency and number of people using the recreational water.

The prioritization or tiering of monitoring locations and sampling frequency should include an evaluation of the recreational waters including an assessment of site-specific information and the potential risk that a bloom at the site would present to human health and the health of animals, including pets, wildlife and livestock. This step-by-step process may be informed by how the water body is used by people, whether pets or livestock may enter it, and other relevant factors. This process will also allow reduction of efforts in locations where blooms are not likely to occur. For example, the Utah Department of Environmental Quality identified 16 sensitive water bodies to monitor closely throughout the swim season, based on the designated uses, the proximity to populated areas, the number of recreational users, and whether cyanobacterial blooms have occurred in the past.

Two EPA documents National Beach Guidance and Required Performance Criteria for Grants, 2014 Edition (EPA 2014a) and Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water (EPA 2015c) provide valuable information on identifying sources of risk and prioritizing resources. The National Beach Guidance and Required Performance Criteria for Grants (2014a) describes steps to take in prioritizing beach monitoring sites based on risk to microbial pathogens and use at beaches. Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water (EPA 2015c) describes the information needed to evaluate drinking water systems' source water vulnerability to HABs.

Step 2: Observe recreational water body for blooms

There are multiple indicators of the potential presence of HABs including visible discoloration of a water body due to suspended cell filaments or scums (e.g., a red, green, or brown tint); thick, mat-like accumulations on the shoreline and surface; foul odors and soupy-consistency of the water; and fish kills. However, toxins can be present at unsafe levels without a visual bloom, and the presence of cyanotoxins can only be confirmed through testing of the water. Cyanotoxin production by cyanobacteria is highly variable and strongly influenced by the environmental conditions as well as the specific cyanobacteria causing the bloom. Microscopic phytoplankton identification can provide information when blooms are not visually apparent and can determine the type of bloom.

Public health officials should encourage the general public to notify their state or local government as soon as they see a bloom. EPA's <u>States Resources</u> site contains an interactive map with cyanobacteria/cyanotoxins resources for each state. For example, Ohio EPA encourages individuals reporting potential blooms to fill out a Bloom Report Form on their website and email the form, with attached digital photographs (if available), to a designated mailbox (Ohio 2014). Individuals are encouraged to report the bloom location, color, size, appearance, and location of nearby public beach or drinking water plant intake(s) (if any), as well as any other available water quality information. The State of New York has a Suspicious Algae Bloom Report Form and a program for citizens to send in photos of the suspected blooms (New York, 2015). For examples of state forms for reporting blooms, see Table 3. For a comprehensive list of HABs programs, please visit the <u>States Monitoring Programs and Information</u> website. Managers, public health departments and states may want to consider creating outreach tools for their community to educate the public on blooms and what to do if they see a bloom. In addition, the <u>Cyanobacteria Monitoring Collaborative</u> has an application (app) to find and study cyanobacteria in waterbodies.

State Organization	Link to Reporting Form for Potential		
	Cyanobacterial Bloom		
California State Water Resources Control Board	Freshwater Bloom Incident Form		
Florida Department of Environmental Protection	Algal Bloom Reporting Form		
Illinois Department of Public Health	Bloom Report Form		
	HAB Human Illness Report Form		
	HAB Animal Illness Report Form		
Indiana State Department of Health	Bloom Report Form		
	HAB Human Illness Report Form		
	HAB Animal Illness Report Form		
Kansas Department of Health and Environment	Bloom Report Form		

Table 3: Selection of State Forms for the Public to Report Cyanobacterial Blooms

State Organization	Link to Reporting Form for Potential	
	Cyanobacterial Bloom	
	HAB Human Illness Report Form	
	HAB Animal Illness Report Form	
New York Department of Environmental Conservation	Suspicious Algal Bloom Report Form	
Ohio Environmental Protection Agency	Bloom Report Form	
Washington State Department of Health	Bloom report site	

2.1 Field and Visual Identification

A bloom may be observed or reported by environmental or public health staff or the general public. Visual inspection can rule out a bloom as cyanobacterial based on the characteristics of the bloom and by performing the "jar" or "float" test. However, toxins can be present without a visual bloom on the water surface, and the presence of cyanotoxins can only be confirmed by testing the water.

The State of Vermont developed a guidance document to help communities within the state address issues associated with cyanobacteria. The guidance document has helpful tips on identifying cyanobacterial blooms and conducting jar (or float) or stick tests (Cyanobacteria Guidance for Vermont Communities) (Vermont 2015).

Microscopic phytoplankton identification can provide information when blooms are not visually apparent and can determine if species present are toxic- or non-toxic species and the type of bloom. For more information on identifying blooms and specific cyanobacteria, go to: <u>USGS</u> <u>Field and laboratory guide</u>, <u>Guidance Document for Harmful Algal Blooms in Colorado</u>, and <u>Guidelines for safe recreational water environments</u> (WHO 2003). Other States such as Oregon, California, and New York have also developed guidance materials, including videos on how to conduct shoreline sampling. In addition, there are academic programs, websites and apps to help with identification of cyanobacteria and blooms, for example, the New England-based Cyanobacteria Monitoring Collaborative. BloomWatch is an app which uses crowdsource data to find and report potential cyanobacterial blooms. Individual citizens who download the BloomWatch app and use it to submit their photos of the potential bloom to the BloomWatch project. Users may also send that information to the relevant state or local agency.

In addition to visual inspection for blooms, recreational water managers may want to consider routine sampling and testing for cyanobacterial cells and/or biomass and cyanotoxins. Testing for cyanobacterial cells is less expensive than testing for cyanotoxins using some analytic methodologies (e.g., mass spectrometric) if taxonomists are already available. Testing can:

- Help distinguish green algae and diatom blooms from potentially harmful cyanobacterial blooms;
- Provide information on cyanobacteria that may be present below the surface, and not visually apparent at the water surface; and,
- Detect lower concentrations of cyanobacteria that may not be visually apparent.

Utah DWQ involves citizens in the monitoring and identification process in addition to reporting blooms. Volunteers are taught how to identify cyanobacteria and in many cases provided with microscopes for family-level taxonomic identification which can be reported to the National Oceanic and Atmospheric Association (NOAA) <u>Phytoplankton Monitoring Network</u>. Monitoring activities are also conducted by local health departments which are often responsible for issuing

the advisories. In addition, many of the monitoring groups use toxin test strips (stick or dip tests) to detect immediate threats. Broadly, this information acts as an initial screen to notify the Utah DWQ when to collect phytoplankton/toxin samples for analysis by professional labs.

2.2 Predictive and Remote Monitoring Tools

New technologies are changing the way we monitor pollution in the environment. EPA and other agencies are studying innovative technologies to support monitoring efforts in assessing water quality, including cyanobacterial blooms and nutrient pollution using:

- Satellites;
- Portable and ground remote sensors; and
- Measurement and model data which can be used in predictive modeling applications.

NOAA satellite imagery data are being used to predict blooms in western Lake Erie and the Gulf of Mexico (Florida and Texas). NOAA, EPA, United States Geological Survey (USGS), and NASA are partnering on the Cyanobacteria Assessment Network (CyAN) project. Once new satellite sensor data are available, this collaborative will make available nationwide satellite data which has been processed for cyanobacteria abundance for all large inland lakes. The goal of the CyAN project is to develop an early warning indicator system using historical and current satellite data to detect cyanobacteria blooms in U.S. freshwater systems. This project supports federal, state, and local partners in their monitoring efforts to assess water quality to protect aquatic life and human health. Satellite imagery may not be appropriate for all waterbodies because most satellites support resolutions (e.g., 30 m - 1 km) that are adequate only for moderate- to larger-sized lakes and usually at a frequency of no more than once a week.

Environmental monitoring of physical, chemical and biological indicators of bloom formation potential is important but can be resource intensive if data are not already available from other sources. Key indicators include cyanobacterial cell counts, biovolumes (the volume of cells in a unit amount of water, mm³/L), chlorophyll *a* and phycocyanin concentrations, presence of cyanotoxin production genes in the water body, nutrient concentrations (nitrogen and phosphorus), changes in hydrophysical conditions and new weather patterns (such as increased temperature and rain) (Izydorczyk et al., 2005; Ohio 2014). EPA encourages recreational water managers to use all available data, as discussed in Step 1, as part of a weight of evidence approach to determine if recent changes have occurred, possibly indicating bloom occurrence. Bloom indicators can be used to inform a decision whether toxin analysis should proceed.

As previously noted, although EPA has draft recommendations for specific cyanotoxins (see Table 1), cyanobacterial cell counts can be informative and serve as an interim step before toxin analysis. WHO established guidelines (see Table 2) and several states (such as Connecticut, Indiana, Kentucky, Oklahoma, Utah, and Wisconsin) use cyanobacterial cell levels for their swimming advisory level.

2.3 Continued assessment of waterbodies for potential blooms

Properly trained staff might conduct field inspections of those recreational waters that are likely to be vulnerable to blooms (as determined in Step 1) focusing on specific seasons the water was previously determined to be vulnerable. For those recreational waters that the state or locality has determined not to be vulnerable to cyanotoxins or blooms, the manager may still want to consider periodically reassessing as watershed characteristics may change over time.

For vulnerable waters, even if there is no indication that a bloom has occurred, EPA encourages the program to continue observing for possible blooms throughout the vulnerable season (determined previously as part of this Step). If any of the observations indicates a bloom is occurring, the manager may want to notify the public by posting cautionary warning signage and begin monitoring for toxins.

2.4 Notification of risk from bloom and potential cyanotoxins

When a bloom and/or the presence of cyanotoxins are confirmed, the manager usually issues a notification (which could either be an advisory or a closure) to raise awareness of the potential risks associated with swimming and other water contact activities. Water body or beach advisories are recommendations to limit swimming or other recreational activities, due to an increased health risk due to contact with or ingestion of the cyanobacteria and/or cyanotoxins. An advisory notification does not, however, officially close the recreational area to the public. Advisories can be based on the simple presence of a bloom even though the predominant species of phytoplankton or presence of cyanotoxins has not yet been confirmed. Alternatively, the advisory may also be posted only after confirmation of the presence of cyanotoxins. Permanent advisories may be used to notify the public of a continuing potential human health risk associated with use of the water. In contrast, a closure notification or posting typically means that the water body is officially closed to the public. Closing a water body or a beach is a local decision. Templates for communication materials are available in EPA's <u>Recreational Water Communication Toolbox</u>.

Several States have risk communication tools to assist during a cyanobacterial bloom event. For example, California has a voluntary guidance system of thresholds, decision tree, and signage used to notify the public regarding freshwater HABs. These materials are posted on the <u>California HABS Portal</u>. California has proposed a 3-tiered approach to notifying the public. If a bloom is suspected based on visual elements (e.g., water appears soupy) or if there is an increase or change in pH or nutrient loading, or other characteristics that historically have predicted blooms, then a "warning" sign is posted. If human or animal illness is suspected, then a "caution" sign is posted. "Danger" is posted once a human illness, or animal illness or death has been confirmed due to cyanotoxin exposure.

Oregon Department of Environmental Quality has developed a harmful bloom strategy (Oregon HAB Strategy) with the goal of preventing and controlling HABs. The voluntary strategy focuses on identifying waters that experience HABs, issuing health advisories and educating the public about their risks. If a bloom is present, the strategy recommends collecting samples for algal identification and cell counts and/or analysis of toxins.

If there is no indication of a bloom, EPA encourages the continued observation of vulnerable waters for possible blooms throughout the season as determined previously as part of this Step (see discussion under section 2.3). If a bloom occurs near where people, pets, or livestock could be exposed, the recreational water manager may want to begin monitoring that water for toxins (Step 3).

Step 3: Monitor for cyanotoxins

3.1 Methods

There is a diverse range of rapid screen tests and laboratory methods used to detect and identify cyanobacteria cells and cyanotoxins in water. Types of methods include:

- Enzyme–linked immunosorbent assays (ELISA);
- Reversed-phase high performance liquid chromatographic methods (HPLC) combined with mass spectrometric (MS, MS/MS) or ultraviolet/photodiode array detectors (UV/PDA);
- Protein phosphatase inhibition assay (PPIA);
- Liquid chromatography/mass spectrometry (LC/MS);
- Quantitative Polymerase Chain Reaction (qPCR) and microarrays/DNA chips; and
- Cyanobacteria cell counts through microscopy.

For more information about these methods, see this EPA website <u>A Summary of the Methods</u> <u>Available for Cyanotoxin Detection</u> and <u>USGS Analytical Methods for Cyanotoxin Detection</u> <u>and Impacts on Data Interpretation</u> (USGS 2010). In addition, see a list of specific methods in Table 4, below.

The decision to use a monitoring method should be made based on the needs and resources of the waterbodies to be monitored. Methods vary widely in sensitivity, rapidity, cost and level of expertise required to perform the method. The potential risk from cyanotoxins can be estimated by directly measuring the toxin (bioassays- HPLC, MS, LC/MS), by measuring antibodies raised against the toxin (bioassays by chromatography - ELISA, PSI, PPIA) or by estimating the potential for toxin production by measuring a gene that can produce the toxin in cyanobacteria (PCR). As demonstrated in studies comparing these methods (Gaget 2017; Loftin 2008) there is no single, best method. Each method has its strengths and weaknesses. For example, some chromatographic methods are very accurate but are limited to one or a few cyanotoxin congeners of a toxin; such methods may underestimate the total risk. PCR methods can be rapid and reliable measures of potential risk; however, if the cyanobacteria are not producing or releasing the toxin, the risk may be overestimated. Managers may also combine methods by adding a confirmation test. Commercially available Enzyme-Linked Immunosorbent Assay (ELISA) test kits are one of the more commonly utilized cyanotoxin testing methods, since they do not require expensive equipment or extensive training to run. Semi-quantitative field screening ELISA kits are available for the presence or absence of cyanotoxins. If cyanotoxins are detected by a field screening kit, repeat analysis is recommended using either a quantitative ELISA test or one of the other analytical methods identified above.

More precise, more quantitative ELISA test kits are available for microcystin-LR, microcystins/nodularins (Adda), saxitoxin, and cylindrospermopsin. In addition, a rapid receptorbinding assay kit is available for the detection of anatoxin-a. Although they provide rapid results, ELISA kits generally have limitations in specificity and are not congener specific. In addition, some cross-reactivity may occur. The microcystins/nodularins (Adda) kit is based on the Adda structure within the microcystin molecule and is designed to detect over 100 microcystin congeners identified to date; however, it cannot distinguish between congeners). Methods that utilize liquid chromatography combined with mass spectrometry (LC/MS) can precisely and accurately identify specific microcystin congeners for which standards are available; LC/MS methods have also been designed to minimize matrix interference. At this time there are only standards for a limited number of the known microcystin congeners. If congenerspecific information is needed, an LC/MS method should be considered. HPLC-PDA methods are less specific than LC/MS methods, and the quantitation is more problematic due to less specificity and sample matrix interference. However, when analytical toxin standards are available for confirmation, they provide a measure of resolution of the congeners present.

For detection of cyanotoxins in drinking water, EPA developed <u>Method 544</u>, a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for microcystins and nodularin (combined intracellular and extracellular), and <u>Method 545</u>, a liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) method for the determination of cylindrospermopsin and anatoxin-a. EPA also developed <u>Method 546</u>, an ELISA Method for ambient and drinking water. Standard operating procedures for this method developed by Ohio EPA provide additional advice for quality-control and sample-handling measures for ambient waters (<u>Inland Lakes Sampling Procedure Manual</u>) (Ohio 2012). For additional information on analytical methods for microcystins and cylindrospermopsin, please also see the analytical methods discussion under Section 5 of the EPA drinking water health advisories (EPA 2015a,b).

EPA's National Aquatic Resource Surveys (NARS) are statistical surveys designed to assess the status of and changes in quality of the nation's coastal waters, lakes and reservoirs, rivers and streams, and wetlands. NARS 2012 and 2016 surveys included testing related to blooms (littoral chlorophyll-*a*, algal toxin, and phytoplankton). The NARS field operation and lab manuals are a good source of information (NARS technical manuals) (EPA 2012a,b)

TEST/ METHOD	Analytical Target	APPROX. LIMIT OF	LOCATION OF	TIME TO	SCREENING
		QUANTIFICATION	TEST	RESULT	ONLY TOOL
EPA Method	Intracellular and	0.10 5.0 μσ/Ι			
546	Extracellular	0.10–5.0 μg/L	Lab	~ 1 day	No
Adda-ELISA	Microcystins				
ELISA-DM	Total Microcystins	0.010 µg/L	Lab	3 hours or	No
Laboratory Test		0.010 µg/L	Lab	less	NO
ELISA	Total Microcystin in			3 hours or	
Laboratory Test	marine/brackish	0.016 μg/L	Lab	less	No
SAES (Abraxis)	water			1633	
ELISA	Total			3 hours or	
Laboratory Test	Cylindrospermopsin	0.05 – 2.0 μg/L	Lab	less	No
(Abraxis)	Cymurospermopsin			1622	
ELISA	Total	0.1-2.0 μg/L	Lab	~2 hours	No
Laboratory Test	Cylindrospermopsin				

Table 4: Analytical methods of Microcystin and Cylindrospermopsin detection and non-toxins in ambient water¹

¹ EPA does not endorse any particular brand or method in this table, and there may be other similar services that have been inadvertently left out of the table or have been developed after the release of this document. Other than EPA Method 546, EPA has not approved or verified these methods.

TEST/ METHOD	Analytical Target	APPROX. LIMIT OF QUANTIFICATION	LOCATION OF TEST	TIME TO RESULT	SCREENING ONLY TOOL
(Beacon)					
HPLC-UV (PDA)	Total Microcystins- limited specificity	~0.3 μg/L	Lab	~ 1 day	No
Protein Phosphatase Inhibition Assay	Total Microcystins	0.02 μg/L	Lab	N/A	No
Microcystin Tube Kit (Abraxis)	Total Microcystins	0.1-5.0 μg/L	Lab	~45 minutes	No
Microcystin Tube Kit (Beacon)	Total Microcystins	0.3-5.0 μg/L	Lab	~1 hour	No
	Total Microcystins	1–10 μg/L	Field	~45 minutes	Yes
Abraxis Test Strip	Total Cylindrospermopsin	0.5–10 μg/L	Field	~45 minutes	Yes
Non-Toxins Methods					
Phycocyanin Continuous Monitoring	Cells/ Biomass	0.04 μg/L	Field	N/A	Yes
qPCR	Cells/Biomass		Lab	~30 minutes	Yes
DNA Chip	Cells/Biomass	Presence/Absence	Lab	~4 hours	Yes
Microscopic Techniques	Cells/Biomass	N/A	Lab	N/A	Yes

In the case of public waterways and drinking water sources, many state environmental agencies operate monitoring, sampling, and testing programs. Several of these states perform the analysis on samples taken from potential blooms in state-run laboratories; however, other states with HAB programs, in addition to municipalities, private utilities, and other stakeholders of freshwater systems send their samples to commercial and public laboratories. States should consider providing training to lab personnel to ensure consistent results. For a non-comprehensive list of laboratories that accept samples for cyanobacteria and cyanotoxin analysis, please visit the State Resources page on this website (<u>States Resources</u>).

If cyanotoxins are confirmed, EPA encourages contacting appropriate partners that use the same water body and alert them to the potential threat as well as contacting managers of downstream recreational areas. EPA recommends that state water recreation managers or appropriate state partners report suspected and confirmed harmful blooms and/or human and animal illnesses associated with HABs to the One Health Harmful Algal Bloom System (OHHABS). The Centers for Disease Control and Prevention (CDC) developed OHHABS as a voluntary reporting system available to state and territorial public health departments and their designated environmental health or animal health partners. For guidance about defining a bloom and how to report health and environmental data, see <u>OHHABS</u>.

3.1.2 Sampling Logistics

Select monitoring sites to ensure that the main public access locations are included, as well as those areas prone to scum build-up due to prevailing winds (e.g., shorelines). Samples should be handled properly to ensure reliable results, whether analyzing the samples using a field kit or shipping them to a laboratory. EPA recommends that a manager follow sample collection and handling procedures required by the method or laboratory performing the analysis. For laboratory analysis, EPA encourages the use of laboratory-provided sample containers to collect water samples. Laboratories may not accept containers from other sources, or they may invalidate results. Amber glass containers are typically used to avoid potential cyanotoxin adsorption associated with some plastic containers and to minimize exposure to sunlight (U.S. EPA, 2014a). Samples should be cooled immediately after collection, during shipping, and pending analysis at the laboratory. Ideally, samples should be shipped on the same day they are collected. Samples generally should be analyzed within five days from the time of collection. EPA encourages systems to contact the laboratory prior to shipping samples for additional sample handling instructions. More information is available from (USGS 2008) Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste-and-Odor Studies in Lakes and Reservoirs.

3.1.3 Safety and handling

Because the water body may contain toxins, samplers should take the following safety precautions. Wear appropriate safety equipment, for example gloves, eye protection (such as goggles), and waders/boots during sampling. Do not ingest water or allow the water to come into contact with exposed skin. Avoid inhaling spray caused by boats, wind or other water surface disturbances. If these conditions are present, wear a mask to avoid inhalation of water spray. Hands should be washed thoroughly after sampling before eating or drinking. Waders/boots should be rinsed before storage. It is important that sampling crews also watch for and report any symptoms of exposure to cyanotoxins, which can occur immediately to several days following exposure. In addition to personal protection, rinsing equipment between uses will help avoid any potential cross-contamination of waterbodies if multiple waterbodies are sampled using the same equipment. See *Recommended Standard procedures for phytoplankton collection to detect harmful algal blooms* (Utah 2016), Chapter 9 of the USGS 2014 National Field Manual for the Collection of Water-Quality Data, and EPA's Sampling Guidance for Unknown Contaminants in Drinking Water (EPA 2017) for further information.

Step 4: Follow up cyanotoxin monitoring

In cases where monitoring (Step 3) is triggered by visual confirmation of blooms, EPA encourages the recreational water manager to continue monitoring and to take notification action(s) until the toxin level is no longer measurable or consistently below the trigger value. EPA recommends, at a minimum, continuing notification actions until at least two consecutive tests show that the toxin level is below the trigger value as was described on page 25 of Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water (EPA 2015).

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