ORSANCO Harmful Algae Bloom Monitoring, Response and Communication Plan
Final

February, 2021

Contents

ORSANCO Role and Responsibilities Summary

Advisories

ORSANCO does not issue advisories for drinking water or recreation. This is the responsibility of the States.

Monitoring

ORSANCO works with the States and Federal partners to quickly identify all reported blooms. The Commission also maintains Harmful Algal Bloom (HAB) monitoring stations and makes visual inspections for algal blooms while conducting other routine monitoring activities.

Response to an Ohio River HAB Event

ORSANCO will coordinate with the States to ensure adequate coverage of on-going HABs.

ORSANCO will serve as a repository for data. The data will be available on our website and updated weekly.

Communications during an Ohio River HAB Event

ORSANCO will convene weekly calls. The purpose of these calls is to:

Discuss sampling results
Coordination of Federal/State/ORSANCO crews for follow up sampling
Identification of laboratories for follow up sample analysis
Identify the need to post or lift advisories

ORSANCO will communicate the sampling results to the state and federal primary contact list (Attachment D), the Technical Committee, ORSANCO's Water Users Advisory Committee, and the general Spills distribution list.

Introduction

The Ohio River Valley Water Sanitation Commission (ORSANCO) is an interstate agency for water pollution control in the Ohio River Basin, representing Illinois, Indiana, Kentucky, New York, Ohio, Pennsylvania, Virginia, West Virginia, and the federal government. ORSANCO's role regarding to Harmful Algal Blooms (HABs) is to monitor the Ohio River and coordinate response to reported HABs.

Algae are a natural component of the Ohio River and are present throughout the year. During optimal conditions some algae may rapidly proliferate causing a "bloom". On the Ohio River the conditions that allow these blooms to occur are typically low and slow flow, and clear and warm water. These conditions give the algae a long residence time, a larger photic zone, and optimal growing temperatures. During a bloom the algal concentration may go from a few thousand cells per milliliter (cells/ml) of water to hundreds of thousands or even millions of cells/ml. Algae blooms are most common in the summer although they may occur at any time of the year.

Sampling on the Ohio River has identified over 300 different species of algae. These algae are divided into 8 taxonomic divisions with the most common being diatoms (Bacillariophyta), green algae (Chlorophyta) and blue-green algae (Cyanobacteria).

Some cyanobacteria can produce toxins (cyanotoxins) which can be harmful if ingested and/or if people or animals come into contact with toxin contaminated water. Cyanobacterial blooms that affect water quality and/or produce cyanotoxins are considered cyanobacterial HABs or HABs.

Some blooms can produce compounds which can cause taste and odor problems for drinking water utilities. During a HAB event, drinkin water treatment managers may need to adjust drinking water treatment approaches to manage for taste and odor concerns and/or the presence of cyanobacteria and/or cyanotoxins in source waters.

According to US EPA the four most commonly found cyanotoxins in the U.S. are microcystins, cylindrospermopsin, anatoxins and saxitoxins (https://www.epa.gov/cyanohabs/learn-about-cyanobacteria-and-cyanotoxins). Each of these cyanotoxins can be produced by several genus/species of cyanobacteria. Lipopolysaccarides in cyanobacteria's cell wall could cause skin irritation if in contact during recreational activities and possible gastrointestinal diseases or intestinal illness if ingested. A list of cyanobacteria genera and the cyanotoxins they produce is in **Attachment A**.

History of HABs on the Ohio River

On August 19, 2015, ORSANCO received a National Response Center (NRC) report of a paint-like green material on the Ohio River at Pike Island Locks and Dam (mile 84.2) which covered 100 X 200 feet. This was quickly identified as the cyanobacteria *Microcystis aeruginosa*. The bloom produced instream levels of the cyanotoxin microcystin as high as 3,000 ug/L. Over the next month this bloom expanded to cover the Ohio River from Pike Island L&D to Cannelton L&D (river mile 84.2 to 720.7). Contact recreation advisories were issued by Ohio, West Virginia, Kentucky, and Indiana. These

advisories remained in place for 2 months. Prior to this event, the largest algal bloom recorded on the Ohio River occurred in 2008 and covered about 30 miles and lasted 10 days.

On September 11, 2019 an algae bloom was identified near Russell, KY. This bloom was identified as *Microcystis wessenbergii* and later included *M. aeruginosa* and *M. flos-aquoe*. This bloom was concentrated in the Greenup, Meldahl, Markland and McAlpine pools but was found intermittently at other locations. Ohio, Kentucky, and Indiana issued recreation advisories which were in place for over a month.

As a result of these events, ORSANCO has undertaken several actions to monitor and respond to HABs including increasing monitoring for HABs, increased cyanotoxin analytical capabilities, evaluation of the data to investigate the causes of the blooms, and the development of the current HABs Monitoring, Response and Communication Plan.

Goals

This guidance document will outline ORSANCOs actions to monitor, predict, identify, and respond to HABs. The goals of these actions are to:

A. Allow the States and health departments to manage the Ohio River's use as a source of recreation.
B. Allow water utilities to use the Ohio River as a source of safe drinking water.

Algae/Toxin Advisories

Some states developed their own guidelines for drinking water and contact recreation. Others adopted USEPA's non-regulatory drinking water health advisories and/or recommended recreation contact/swimming advisories for the cyanotoxins microcystins and cylindrospermopsin. This has caused both drinking water and contact recreation guidelines to vary from state to state. The tables below summarize these guidelines for the states that border the Ohio River as well as USEPA's.

Drinking Water

In May 2015 USEPA issued Drinking Water Health Advisory Levels for two cyanotoxins, microcystin and cylindrospermopsin. These advisories are considered protective of non-carcinogenic adverse health effects over a ten-day exposure to these toxins in drinking water. Most states have incorporated these into their own guidelines (see Tables 1-4 below). Ohio and West Virginia include threshold levels for Anatoxin *a* and Saxitoxin.

Table 1: Drinking Water Advisory Levels for Illinois, Indiana, and Kentucky

Drinking Water Thresholds	Drinking Water Health Advisory (10-day)	
	Microcystins (ug/L)	Cylindrospermopsin (ug/L)
Bottle-fed infants and pre-school children	0.3	0.7
School-age children and adults	1.6	3.0

Table 2: Drinking Water Advisory Levels for Ohio

Drinking Water Thresholds	Microcystins (ug/L)	Anatoxin a (ug/L)	Cylindrospermopsin (ug/L)	Saxitoxins (ug/L)
Do Not Drink – children under 6	0.3	0.3	0.7	0.3
Do Not Drink- children 6 and older and adults	1.6	1.6	3.0	1.6

Table 3: Drinking Water Advisory Levels for West Virginia

Drinking Water Thresholds	Microcystins (ug/L)	Anatoxin a (ug/L)	Cylindrospermopsin (ug/L)	Saxitoxins (ug/L)
Do Not Drink – children under 6	0.3	20	0.7	0.3
Do Not Drink- children 6 and older and adults	1.6	20	3.0	1.6
Do Not Use	20	300	20	3

Pennsylvania has not promulgated any regulatory water quality criteria for any cyanotoxins or implemented any advisory levels. USEPA has issued drinking water health advisory levels for two cyanotoxins, which are the levels shown in Table 1. As noted on that EPA website (https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins), these drinking water health advisory levels "are not regulations and should not be construed as legally enforceable federal standards." PADEP's Safe Drinking Water Program is developing a Standard Operating Procedure that will provide additional details as to what corrective actions may need to be implemented by public water systems when a Health Advisory level is exceeded in the finished water. Generally, if a water system detects microcystins or cylindrospermopsin at levels above the EPA drinking water HA's for more than 10 days, SDW will require the PWS to implement corrective actions (including Public Notice).

Contact Recreation

In May, 2019 USEPA published Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin (Table 5).

Table 4: Recreational Advisory Levels for USEPA, and Illinois.

	Microcystins (ug/L)	Cylindrospermopsin (ug/L)
Human Recreation	8	15

Table 5: Recreational Advisory Levels for Kentucky.

	Microcystins (ug/L)	Cylindrospermopsin (ug/L)	Anatoxin a (ug/L)
Human Recreation	8	15	80

Table 6: Ohio and West Virginia Guidelines for Cyanotoxins in Recreational Waters

Threshold	Microcystins* (ug/L)	Cylindrospermopsin (ug/L)	Anatoxin-a (ug/L)	Saxitoxins* (ug/L)
Recreational Public Health Advisory	8	15	8	0.8

*Microcystins and Saxitoxins thresholds are intended to be applied to total concentrations of all reported congeners/variants of those cyanotoxins.

Table 7: West Virginia Guidelines for Cyanotoxins in Recreational Waters

Threshold	Microcystins* (ug/L)	Cylindrospermopsin (ug/L)	Anatoxin-a (ug/L)	Saxitoxins* (ug/L)
Informational Sign	<6	<5	<80	<0.8
Recreational Public Health Advisory	6	5	80	0.8
Recreational No Contact Advisory	20	20	300	3

*Microcystins and Saxitoxins thresholds are intended to be applied to total concentrations of all reported congeners/variants of those cyanotoxins.

Table 8: Indiana guidelines for Algal Toxins in Recreational Waters

Exposure Reference Value	Microcystins (ug/L)	Cylindrospermopsin (ug/L)	Anatoxin-a (ug/L)	Saxitoxins (ug/L)
Human Recreation Advisory	8	15	80	0.8
Human Recreation Prohibited	20	20	300	3
Dog Recreation Prohibited	0.8	1	Any detection	Any detection

Pennsylvania has not formally adopted contact recreation criteria for cyanotoxins, however, the Pennsylvania HAB Task Force recommends the following cyanotoxin thresholds for human primary contact recreation:

Table 9: Pennsylvania recommended thresholds

Response level	Microcystins	Anatoxin-a	Cylindrospermopsin	Saxitoxins
Advisory (Yellow)	8.0 *	80 †	15 *	0.8 †
Avoid Contact (Red)	20 †	300 †	20 †	3.0 †

* recommended values from USEPA 2019

† thresholds from the State of Ohio 2016 HAB Response Strategy for Recreational Waters (see Table 2)

The World Health Organization (WHO) published guidelines (1999) based on health effects for determining the severity of a bloom (Table 6). While these are not incorporated into criteria, some states use these to trigger increased sampling.

Table 10: WHO Guidelines for HABs in Recreational Waters

Guidance Level	Concentration	How Guidance Level Derived	Health Risks
Low probability of health effects	20,000 cells/ml or 10 ug/L of chlorophyll *a* with cyanobacteria dominant	Human bathing epidemiological study	Short term- skin irritations, gastrointestinal illness
Moderate probability of health effects	100,000 cells/ml or 50 ug/L of chlorophyll *a* with cyanobacteria dominant	Provisional drinking water guideline value for microcystin and other cyanotoxins	Potential for long term illness as well as short term health effects
High probability of health effects	Cyanobacteria scum formation in areas where whole body contact occurs	Inference from oral animal lethal poisonings and human illness case histories	Potential for acute poisoning

These guidelines and advisory concentrations are provided for information purposes only. ORSANCO will defer to the individual states to determine if a drinking water or recreation advisory needs to be reported.

Advisories

ORSANCO's role is to provide timely information so that State resource managers can advise the public concerning use of the Ohio River as both a source of drinking water and for recreation.

ORSANCO will not issue recreation advisories for the Ohio River. Recreation advisories will be issued by each state, typically through their local health departments. ORSANCO will refer any questions from local health departments to the appropriate State Department of Health.

ORSANCO will not issue "Do Not Drink" advisories for drinking water. These will be issued by each utility in coordination with the appropriate state, typically through their State Department of Health. ORSANCO will refer any questions about "Do Not Drink" advisories to the appropriate State agency.

ORSANCO will provide a link on its website to each state's public information concerning HABs on the Ohio River.

Monitoring

Algae blooms may occur anywhere on the Ohio River and any time during the year. However, HABs most often occur during low flows and elevated temperatures. The primary months for HABs are July-September.

Following is a list of methods for identifying indicators of potential algal blooms or cyanotoxins which will trigger investigation/sampling by ORSANCO field staff.. Any potential algae blooms being investigated will be reported to the State HAB contacts (Attachment D).

Direct Observation

ORSANCO personnel will observe sections of the river as part of their normal sampling routine. These areas vary as biological survey crews monitor 3 different pools each year. Crews also conduct water quality sampling at fifteen mainstem and fourteen tributary locations in July and September.

During the July-September period ORSANCO will make a monthly request from all Ohio River water utilities for the current conditions of their raw water (temperature, pH, and turbidity data), as well as notes on any indicators of a bloom (i.e. taste and odor complaints, increased use of carbon treatment, decreased filter run times, increased chemical needs/usage, difficulty in maintaining finished water residual chlorine, meeting turbidity goals or visible algae scums on the river). We will also ask for timely notification of any noted algal problems during the month. For those utilities with greater capabilities we will ask for cyanotoxin concentrations, and chlorophyll/algae concentrations.

ORSANCO will also work with Dam Operators (US Army Corps of Engineers) and other public river users to identify potential HABs.

A guide to identifying HABs will be provided to Dam Operators, water utilities, and other interested parties.

Datasondes

ORSANCO operates 4 datasondes with telemetry systems located at Pike Island L&D, Meldahl L&D, Markland L&D and Newburgh L&D. Sensors on these datasondes collect pH, conductivity, turbidity, dissolved oxygen, temperature, and chlorophyll *a* data every 30 minutes. The telemetry systems allow the data to be available in real-time from ORSANCO's website.

Satellite Imagery

Satellite imagery from LANDSAT and OLCI are available to identify areas of increased algal growth. Because of the temporal nature and spatial extent of HABs, satellite imagery will be a valuable tool to help focus limited resources on areas of probable HAB development.

These satellites provide images at varying geographic and temporal scales, although cloud cover may impact the usability of the images.

When areas of high chlorophyll-a are identified, ORSANCO will coordinate with state and local personnel to investigate.

Response

Sampling

To assist the states in managing the Ohio River, ORSANCO will identify HABs and determine bloom extent. ORSANCO will coordinate with the states to ensure continued monitoring of on-going HABs.

Samples will be screened using in-house resources both to identify the type of algae (i.e. green algae vs. cyanobacteria) and the presence/absence of microcystins and cylindrospermopsin.

Samples collected for making management decisions (e.g. recreation advisories) will be sent to a certified laboratory identified by the affected states.

Final data will be available on ORSANCO's website with weekly updates. This data is provided to state and federal agencies as well as drinking water utilities.

ORSANCO uses the Ohio EPA Standard Operating Procedures for sampling Recreational Waters as applicable (see **Attachment B**).

Analytical Methods

Two methodologies are currently used to analyze for cyanotoxins- ELISA methods (Enzyme-Linked Immunosorbent Assay) and liquid chromatography (LC-MS/MS). Most States use the Abraxis ELISA system with some preferring LC-MS/MS. For rapid turnaround of samples ORSANCO uses the MBio ELISA system. See **Attachment C** for a comparison of the methods.

Communication

Communication and coordination are the primary roles of ORSANCO during a HAB event. This section will delineate ORSANCO's responsibilities to its stakeholders. The Contact List in **Attachment D** denotes the Primary Contact, Health Department Contact, and Public Information Contact for each state and federal agency.

When an algae bloom is confirmed on the Ohio River ORSANCO will inform the Primary Contacts and Water Users Advisory Committee (WUAC), whether it is a HAB. When a HAB is confirmed ORSANCO will convene a weekly conference call with the Primary Contacts, WUAC, and Health Department Contacts. These organizations will be responsible for including others from their organizations (e.g. sampling personnel). The purpose of this call will include, but not be limited to:

- Discuss sampling results
- Coordination of state/ORSANCO crews for follow up sampling
- Identification of laboratories for sample analysis
- Identify the need for advisories

Separate calls to subgroups may be used as needed (e.g. Public Information Officers to coordinate press releases)

Sample Results

In general, data that is collected by ORSANCO is available to the public. Cyanotoxin analytical results are often reported to ORSANCO by the laboratories prior to being confirmed. These preliminary results are not available to the public. Typically confirmation of the results is received within 2-3 days at which point it will become publicly available.

The preliminary data may be used by ORSANCO and state/federal resource managers to make decisions regarding the response to the HAB. ORSANCO will gather all available State and Federal generated data and act as the repository. This will allow easier decision making by the resource managers. ORSANCO will provide access to this data through a password protected website.

Commissioners

ORSANCO will provide a weekly update of on-going HAB events to the Commissioners.

Water Utilities

ORSANCO will inform the WUAC and the general Spills list when algae blooms are identified on the Ohio River, whether these blooms are HABs or not. During a HAB event ORSANCO will provide weekly updates to these groups regarding sample results and extent.

Drinking water system advice and communication related to treatment will be done by the appropriate State Environmental Agency to the individual water systems.

Media Contact

Media contacts will be handled by ORSANCO by providing general information. Specific questions about advisories or the impact of sample results on advisories will be referred to the Public Information Office (PIO) contact for each State Environmental or Health Agency. General information for media contacts is available from USEPA at https://www.epa.gov/cyanohabs.

General Information

A number of Federal, State or Local agencies and NGO's may ask for updates on a HAB event. ORSANCO will provide weekly email updates of on-going HAB events to these groups as they are identified. Communication with the general public regarding risks/advisories will be forwarded to the appropriate State.

Resources

ORSANCO
http://www.orsanco.org/harmful-algae-blooms

Illinois
https://www2.illinois.gov/epa/topics/water-quality/monitoring/algal-bloom/Pages/default.aspx.

Indiana
www.algae.in.gov

Kentucky
https://eec.ky.gov/Environmental-Protection/Water/Monitor/Pages/HABS.aspx
Ohio
http://epa.ohio.gov/habalgae.aspx#147744472-basics

Pennsylvania:
www.dep.pa.gov/OurCommonWealth/pages/Article.aspx?post=44

www.health.pa.gov/topics/envirohealth/Pages/HABs.aspx

West Virginia:
http://www.wvdhhr.org/oehs/public_health/blue_green_algae.asp

US EPA
https://www.epa.gov/cyanohabs

Centers for Disease Control
http://www.cdc.gov/nceh/hsb/hab/default.htm

US ACE
Louisville District: http://www.lrl.usace.army.mil/Missions/CivilWorks/WaterInformation/HABs.aspx
Pittsburgh District: http://www.lrp.usace.army.mil/Missions/WaterManagement/WaterQuality.aspx

ATTACHMENT A
List of Toxic Algae

Toxin and Taste-and-Odor Producing Cyanobacteria (list is not exhaustive)

(LYN, lyngbyatoxin-a; APL, aplysiatoxins; LPS, lipopolysaccharides; CYL, cylindrospermopsins; microcystins, MC, π-anaGVGBVtoxins; BMAA, β-N-methylamino-L-alaine; NEO, neosaxitoxins; SAX saxitoxins; GEOS, geosmin)

Cyanobacterial Genera	Dermatoxins			Hepatotoxins			Neurotoxins				Tastes and Odors	
	LYN	APL	LPS	CYL	MC	NOD	ANA	BMAA	NEO	SAX	GEOS	MIB
Colonial/Filamentous												
Dolichospermum			X	X	X		X	X	X	X	X	
Anabaenopsis			X		X							
Aphanizomenon			X	X			X	X	X	X	X	
Aphanocapsa			X		X							
Cylindrospermopsis			X	X				X		X		
Fischerella			X					X			X	
Haplosiphon			X		X							
Hyella			X								X	X
Lyngbya (Plectonema)	X	X	X	X				X		X	X	X
Microcystis			X		X			X				
Nodularia			X			X		X				
Nostoc			X		X			X			X	X
Oscillatoria (Planktothrix)	X	X	X		X		X	X		X	X	X
Phormidium			X				X	X			X	X
Pseudanabaena			X		X							X
Raphidiopsis			X	X			X					
Schizothrix	X	X	X									
Umezakia			X	X								
Unicellular												
Synechococcus			X		X			X			X	X
Synechocystis			X		X			X				

Table courtesy of Jennifer Graham, USGS

Please note, some newer data suggests that Aphanizomenon may be capable of producing microcystins.

ATTACHMENT B
OEPA Sampling SOP

HAB SAMPLING IN RECREATIONAL WATERS

This sampling protocol is designed to be responsive to HAB reports in recreational waters so that public health may be protected. It is applicable to collections by anyone who wishes to characterize phytoplankton and cyanotoxins in Ohio waters.

Safety Precautions

Safety must come first when sampling HAB toxins. Shoulder-length gloves should be worn when sampling HABs. Goggles should be worn to prevent spray from getting into the eyes. Chest waders should also be worn if collecting a cyanotoxin sample when wading off the shore to protect skin from contact with toxins. A personal floatation device should be worn. Avoid inhaling spray from boats, wind, or irrigation water from areas with harmful algal blooms. Wear a mask to prevent inhalation of spray.

Do not ingest or allow the water to come in contact with the skin. Always wash hands with clean, fresh water after sampling and do not touch hands to mouth or other exposed areas of the body before washing. All equipment, gloves, and waders should be rinsed with de-ionized water (not lake water) after collections.

Sampling Methodology Goal

The concept behind the sample collection procedures is to focus on collecting samples representative of areas being sampled. Higher cyanotoxin concentrations are expected near shore, especially on the downwind (away from where the wind is coming from) side of a lake. Highest cyanotoxin concentrations are usually expected with scums (below the dead material at the surface), and within dense cyanobacteria blooms. Most cyanobacteria that produce cyanotoxins hold them within their cells and release the toxins upon cell death.
Higher cyanotoxin concentrations may be detected after a rapid bloom die-off, such as when algaecide is added to a dense bloom of cyanobacteria producing cyanotoxins.

Sample Location(s)

Observe areas of likely recreational use or contact. Consider wind direction and where the blooms may be blown such as the downwind side of a lake, or transported by currents. Review any satellite data, if available, to see where the heaviest concentration of cyanobacteria is located. Look for areas of bloom growth and decay throughout the photic zone.

Sample Frequency

Sampling will occur on a case-by-case basis depending on current water conditions. Sampling will focus on the peak recreational season between Memorial Day and Labor Day. If a HAB is identified and verified, a sample will be taken. Continued monitoring may occur beyond the peak recreational season based on environmental conditions and relative health risk, in consultation with Ohio EPA, ODH and ODNR.

Preparations

Plan weekly sampling early in the week and ship for overnight delivery. Microcystin/Cylindrospermopsin samples must

be analyzed within 5 days of collection, and Saxitoxin must be analyzed within 6 days of collection, and all toxin samples must be kept cold and in the dark until laboratory processing. Phytoplankton samples should be preserved with Lugols iodine within 8 hours of collection and kept on wet ice or ice packs, but not frozen.

IMPORTANT - On the Friday before sampling occurs, if shipping to the Ohio EPA DES Laboratory for analysis, contact the DES Sample Coordinator at (614) 644-4243 and indicate how many samples will be collected and when they will be delivered to DES. Be sure to contact the DES sample coordinator with any questions before shipping.

Label Information

Label the collection containers with a waterproof marker or attach a label to the outside of the container and mark with a waterproof marker. Include the following information:

Site Name
Date
Time
Preservative (if applicable)

Sample Collection

Beaches

Phytoplankton Sample Collection at Beaches

The purpose of collecting phytoplankton samples is to identify the organism to determine if the bloom consists of cyanobacteria or another organism. If it is not a cyanobacteria bloom, then no Algal Advisory sign would be posted. If the bloom is cyanobacteria, then the type of cyanobacteria will determine which cyanotoxins should be analyzed.

If the location of the bloom is evident (i.e. at the surface or just below the surface), collect a grab sample from the densest part of a bloom. The grab sample should be collected in a 125 ml graduated glass jar or other Ohio EPA-approved container. If the bloom is not at a distinct location, but diffuse throughout the water column, use a composite sampler that includes a collection for a range of depth. If collecting a scum, collect a grab sample from the scum-water surface interface.

If you suspect the presence of benthic cyanobacteria, collect a sample near (at 1 foot above) the bottom where you believe the benthic cyanobacteria is located.

Ideally samples should be preserved at the time of collection with Lugol's iodine solution at a ratio of 1:100 although Lugol's can be added to a sample anytime within eight hours. To achieve a 1:100 ratio add about 1 ml of Lugol's solution per 100 ml of sample. (Final Lugol's solution in a sample should be 1%.) Final preserved sample color should be similar to that of weak tea. Ship for delivery to the laboratory, such as Ohio EPA's DES. Samples should be kept on wet ice and in the dark during transport. **Do not freeze the phytoplankton sample - doing so will make identification difficult.**

Cyanotoxin Sample Collection at Beaches-Overview

The purpose of collecting cyanotoxin samples is to determine if a Public Health Advisory or No Contact Advisory (if there are probable human illnesses or pet deaths) should be posted.

Samples will be collected from nine locations within the designated recreational area and composited. The nine locations will be determined by evenly dividing the recreational area into three transects that begin at the beach and extend into the water. Samples will be collected from three locations (ankle, knee and hip deep) along each transect. (Note: use a rod ahead of where you are walking to gauge depth. Do not stir up the sediment. If the depth drops off quickly past hip depth, then just collect the ankle-depth and knee depth samples. Do not go past hip depth.)

Wade slowly (as not to stir bottom substrate) to the sampling locations. Avoid collecting suspended sediment that may be kicked up while accessing the sampling point. Ankle-deep water samples will be collected approximately 15 cm below the surface. Knee- and hip-deep water samples will be collected approximately 30 cm below the surface. If dense cyanobacterial accumulations are present outside of the transect locations (which includes a scum or heavy biomass in the photic zone), an additional sample will be collected from the densest accumulation by filling a separate clean 125 ml PETG container or other Ohio EPA-approved container. Submit this sample in addition to the composited samples with a separate Sample Submission Form and clearly marked as scum (adapted from USGS, 2008).

Cyanotoxin Sample Collection Instructions

Use a clean 1-quart Cubitainer™ or other Ohio EPA approved container to collect from each sampling point along all three transects at a beach location. Carry the clean bucket with you (or you can place a float around the bucket). Fill the 1-quart Cubitainer™ or other Ohio EPA- approved container from the ankle-depth location on the first transect and completely dispense the collection into the bucket. Carefully wade out to the knee-depth location with the bucket and collect another 1-quart sample using the same Cubitainer™ or other Ohio EPA-approved container. Completely dispense the sample into the bucket. Then wade out to hip depth and collect another 1-quart sample and completely dispense the collection into the bucket.

Go to the second transect. Using the same 1-quart Cubitainer™ or other Ohio EPA-approved container, collect the three samples along the second transect in the same way the samples were collected along the first transect and dispense them into the bucket with the first transect collections. Once the second transect collections are dispensed into the bucket, go to the third transect and collect the three samples along the third transect in the same way collections were made on the first two transects and dispense into the bucket.

1) Use a clean stirring rod to mix the composite samples from all three transects in the bucket. Continue to stir the composite sample while you dispense a sub-sample of the composite sample into a 125-ml PETG container or other Ohio EPA-approved container This is the sample you will submit to the laboratory.

2) In addition, if a scum is found at any area where the public is expected to recreate outside the transect lines, collect a surface grab sample which includes the scum at the scum-water interface and clearly note the location (coordinates or point on map)and collection type (grab) on the container label. Note the percentage of recreational area covered by the scum on your Sample Submission Form. This sample is **not** mixed into the composite sample but submitted to the laboratory in addition to the composite sample.

3) Immediately transfer each capped sample to a dark cooler on wet ice or ice packs when collected. The sample must be kept in the dark and cool to preserve any toxin that may be present.If there are multiple beaches on a single lake with cyanobacteria blooms, all beaches should be sampled in the same manner as stated above, differentiating each sample location by an alternate location name. When you move to a new beach location to set up new transects, rinse the collection bucket and stirring rod three times with lake water at each location. Rinse away from the transect sampling points so as not to cross contaminate or mix the water where samples will be collected. Use a new, clean 1-quart Cubitainer™ or other Ohio EPA- approved container for each beach to collect samples along the transects and a clean 125ml PETG collection container for the sample taken from the bucket after mixing.

Cyanotoxin Preservation Instructions

Upon collection, samples should be immediately put in a cooler in the dark and on wet ice. If a sample will not arrive for processing at the laboratory within 5 days, the sample must be frozen in a standard freezer until it is processed.

Phytoplankton Preservation Instructions

Ideally samples should be preserved at the time of collection with Lugol's iodine solution at a ratio of 1:100, although Lugol's iodine can be added to a sample anytime within eight hours. Addition of Lugol's iodine will allow for extended preservation.

Equipment Decontamination Between Sampling Locations

When sampling for phytoplankton or cyanotoxins at different contact recreational areas, use clean collection containers. Rinse the collection bucket and stirring rod three times with lake water at each location. Rinse away from the transect sampling points so as not to cross contaminate or mix the water where samples will be collected.

Toxin Processing Instructions

Total toxin (free toxins and endotoxins) shall be determined for recreational water sample analysis. Samples will be processed to ensure all algal cells are lysed, which should be verified through microscopic observation. Freeze/thaw three times is a preferred method for lysing algal cells providing total lysis is confirmed through microscopy.

QA/QC

Ohio EPA will use quality assurance/quality control procedures that meet quality objectives for HAB sampling.

Paperwork

Fill out a Chain of Custody Report and Sample Submission Forms (one for each sample) (see attached templates in Appendix E). Put the paperwork in double ziplock-type bags and seal each bag well. Place the paperwork on the samples in the cooler.

Shipping

Contact the appropriate laboratory prior to collecting samples (see Appendix D). Include any paperwork required by

the receiving laboratory. This will usually include a Chain of Custody Report and a Sample Submission Form (see Appendix E.) Make sure that the data are reported back to the sample submitter and to the DSW HAB Coordinator so that data can be entered into the HAB database.

Enclose each sample container in a separate sealed plastic bag. Place on ice in a sealed plastic bag and place in the shipping container. Note that ice packs should be used if shipping Fed-Ex and wet ice sealed in plastic bags or ice packs for UPS shipments. Prepare the package for shipment.

ATTACHMENT C
Analytical Method Comparison

Analytical Methods Comparison	
MBio Diagnostics	• ELISA method • Field portable • Single sample • 8 microcystin congeners + cylindrospermopsin • Mc detection limit 0.4 ug/L • Results in 10 minutes • $4,000 per system/$20 per sample
Abraxis ELISA	• Separate kits for microcystin and cylindrospermopsin • Not field portable • Mc detection limit 0.15 ug/L • Results in hours • 10+ samples at once • Detects 11 congeners of microcystin at varying recovery rates • Cyanotoxin Automated Assay System (CAAS) greatly increases speed and reduces human error • $30,000 with CAAS/$500 per 96 well plate (10+ samples)
LC MS/MS	• Cost $200,000+ • Significant staff training • Method detects 7 congeners at near 100% recovery • Mc detection limit 0.25 ug/L • Not field-portable • Preferred by State of Kentucky

ATTACHMENT D
State and Federal Contacts

	Public Information	Primary Contact	Health Department
Illinois	Kim Biggs IL EPA 217-558-1536 kim.biggs@illinois.gov	Gregg Good Gregg.Good@illinois.gov 217-782-7082 Tony Dulka ANTHONY.DULKA@Illinois.gov Nicole Vidales Nicole.Vidales@Illinois.gov Teri Holland Teri.Holland@Illinois.gov	Justin Albertson
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