Oregon Harmful Algae Bloom Surveillance (HABS) Program

Sampling Guidelines:
Cyanobacterial Blooms in Recreational Waters

Oregon Health Authority

Public Health Division
Center for Health Protection
Environmental Public Health Section

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Table of Contents

Appendices

These sampling guidelines represent a consensus view among experts and stakeholders involved in CyanoHABs issues either through public health protection, resource management, research, or environmental regulation.

The guidelines were reviewed and approved by laboratories that specialize in cyanobacteria analysis. In any case, contact the laboratory prior to sampling to confirm sample collection and preservation requirements.

Background

Cyanobacteria, also known as blue-green algae, are commonly found in many fresh and salt water environments around the world. Some cyanobacteria species are referred to as toxigenic because they have the potential to produce toxins that can harm people, pets and wildlife.

The Oregon Health Authority, Public Health Division (OHA) is working to gain a better understanding of the occurrence of cyanobacterial blooms (cyanoHABs) in Oregon and their impact on human health. Funding for Oregon's Harmful Algae Bloom Surveillance program was through a five-year federal grant from the U.S. Centers for Disease Control and Prevention (CDC). That grant ended in September of 2013, leaving the program without funding. OHA, however, continues to implement the highest priority activities, such as issuing and lifting advisories, and educating the public about cyanobacteria and cyanotoxins.

Part of the cooperative agreement with the CDC included building the capacity of water body management agencies to monitor water bodies in a scientifically sound manner with the goal of protecting public health. The OHA continues to rely on data from environmental samples collected by water body managers, and other agencies and groups, to inform advisory decisions. OHA works in partnership with management agencies when issuing and lifting public health advisories.

Purpose of this document

The purpose of this document is to provide guidance for managers who intend to monitor freshwater bodies when potentially harmful cyanobacterial blooms are detected. Topics covered include; proper sample collection, cyanobacteria identification and enumeration, and cyanotoxin analysis with the objective of protecting public health, specifically with respect to general recreational uses. It is not intended to cover sample collection for environmental or ecological assessment, or for community water system protection.

Sampling guidance

Assessing the public health risk posed by toxic cyanobacteria or the potential for development of cyanobacterial blooms is complex, and bloom configuration and cyanotoxin production can change rapidly. When deciding to sample an affected water body, consider the following.

Visual Assessment: A Precursor to Sampling

Simple visual assessment is an important tool in recognizing cyanobacteria and cyanobacterial blooms. Visual assessment of bloom status such as areas of discoloration or surface scum collection should be used to guide sampling. Materials and equipment to help identify cyanobacteria (e.g., field guides and microscopes) provide an early warning mechanism to help address concerns about cyanoHABs.

Visual Assessment Steps:

- Determine if a bloom is present based on water color and level of cyanobacteria visible on the surface and/or suspended throughout the water column.

- Describe the location where concentrated cyanobacteria or floating mats are found (e.g., in and around swimming areas).
- Take photos (close-up and overview) of areas with concentrated cyanobacteria or floating mats.
- Record results of the visual assessment on a field data sheet (example in Appendix A).
- Contact the OHA if a bloom is present.

Sample collection

Monitoring should focus primarily on the protection of human health and secondarily on the health of pets and livestock. Visual observation of a cyanobacterial bloom is the precursor for water sampling and is also a key consideration when determining optimal sample locations.

Assessing potential hazards at recreational water bodies can be complicated if there are numerous access points that allow people and animals to enter or move around the water. Important factors to consider when managing a bloom are:

- Cyanobacteria often accumulate along shorelines or structures such as docks and dams, and can be seen as floating mats
- The characteristics of a cyanobacterial bloom can change rapidly due to wind and other environmental or physical factors (waves, currents, etc.)
- Cell densities can also change quickly as cyanobacteria multiply

Scums or thick concentrations of cells in the water column can generally be assumed to present the greatest risk to recreational bathers and are therefore the best places to sample.

Samples should represent worst-case conditions in areas where people and animals are most likely to be exposed to affected water. The location and number of samples will depend upon the recreational use at the water body or near the bloom, and the funding available for sampling and analysis. If funding is limited, sampling should focus on near shore waters in areas where swimming and water play might occur. This is especially important because children and pets often found in these areas are the most vulnerable. If resources allow, sampling far from shore may be desirable to assess risks to water skiers and other deep-water users.

The time of day when samples are taken can be as important as where they are taken. Variations in environmental conditions throughout the day can change the way cyanobacteria react and where you may visually see the bloom. As temperatures rise, cyanobacteria will move closer to the surface creating a scum or appearing as globs of cells in the water column. Wind and underlying current can also change throughout the day, influencing where the bloom may be found and how well-mixed the water will be. Mixing causes the cells and toxins to be dispersed, potentially reducing the number of cells or amount of toxins present in the sample. When planning a sample collection, keep in mind what time of day you would expect the water to be warm and calm, and when peak recreational water activities typically occur. This information will inform your decision as to when to sample a bloom.

Follow-up samples

Each bloom is unique, so the timing of follow-up sampling will vary according to the characteristics of the bloom and the affected water body. Considering the questions below may influence the decisions made about when to collect follow-up samples.

- *Are you conducting Toxin-Based Monitoring: Option 4 (page 6)?* If so, and toxin concentrations are below recreational use values (RUVs, sample every other week.
- *Are there changes in the blooms appearance (e.g. size, location, density, color)? Conduct visual observations while the bloom is present. If toxins were below OHA RUVs and the blooms appearance changes drastically, follow-up sampling is recommended to determine if toxins have spiked.* .
- *Has there been a report of an animal death or human illness?* Such reports are an important indication that further evaluation, such as toxin analysis, should be done.
- *Does the weather forecast call for rain?* Periods of cool rainy weather may contribute to the collapse of the bloom or run-off may introduce nutrients that can lead to the resurgence of a bloom, especially if warm weather follows the rain.
- *Is the wind or current strong enough to move the bloom from the initial sampling location?* Shifts in wind direction can move a bloom from one location to another causing blooms to accumulate along shorelines, structures or in protected areas.
- *Is the temperature rapidly falling?* Cyanobacterial blooms generally do not thrive during the winter months due to low water temperatures. Some genera of cyanobacteria can sink into sediment and become dormant until weather conditions become optimal.

Standardized collection methods

Standardized collection methods ensure samples are representative of field conditions and minimize sample collection variability while meeting program goals and managing limited resources. They also represent a worse- case exposure scenario when people and animals recreate in an affected water body.

The extent of any recreational use advisory depends upon the characteristics of the water body being sampled and the level of monitoring being performed. Depending upon the size, geography, current, etc., OHA staff may determine that the advisory should apply to either the entire waterbody, or if selected areas of the water body can be singled out.

1. *Grab/single point sampling.* This is the most basic collection method used to characterize a bloom. Samples are taken at the point in the bloom that appears to be the most dense or worse-case. If resources permit, additional grab samples can be taken in other areas to determine what portions of a water body are of concern. All results from samples taken at a given water body are considered in the public health advisory process.
2. *Composite sampling*. This method minimizes the most sample variability. because it employs a variety of techniques to combine samples taken at a particular point across

time, at many depths in a single location, or at a given depth in multiple locations. Use the following guidelines when performing composite sampling:

- Take three samples that reflect worse-case conditions (areas with the most scum or thickest cyanobacteria) and combine
- Do not combine samples taken from within the bloom with those taken outside the bloom (in a "clean" area). This will avoid dilution of the cells and toxins and the risk of a false negative during analysis
- Do not combine samples from different blooms or different water bodies as they are likely to have significantly different species composition and toxin profiles.

Appendix B has a list of field equipment and step-by-step instructions for collecting the type of water samples outlined above.

Note: When determining worse-case scenarios, think of the risk of exposure to people and pets through recreational activities. Exposures increase depending upon the type of water activities enjoyed. For people, swimming, active water play, or waterskiing have the greatest potential risk. For animals, swimming, drinking the water, active water play, and eating or licking cells from rocks or their fur have the greatest risk.

Safety

Cyanobacteria can produce potent neurotoxins and hepatotoxins (liver toxins) so exercise care when collecting water samples. In addition, cyanobacteria produce lipopolysaccharides that can irritate the skin. Wear protective clothing such as hip waders and long rubber gloves.

Do not ingest surface water and avoid inhalation of aerosolized water (e.g., created by a boat motor). Always wash hands, other exposed skin (feet, legs, etc.), glasses, boots and gloves with soap and water after collecting samples. The overarching concern when sampling is personal safety. When selecting sampling locations, look for ease of access and make sure there are no physical barriers to safety.

Contact the laboratory you plan to have analyze the sample you collect and inquire about how to preserve the sample. They will either send you the materials to preserve the sample, or they can tell you where to obtain the Lugol's solution. Lugol's solution is used to preserve samples when cell enumeration and species identification is necessary or desired. When using/handling Lugol's, wear protective gloves and eye protection. Add it to the sample(s) in a well-ventilated area such as outside or under a fume hood. Lugol's is commonly used for short-term storage of cyanobacteria- from a few months to a year or more. Refer to the Material Safety Data Sheet (MSDS) for more information regarding the hazards of this chemical and how to protect yourself from exposure.

Toxin-Based Monitoring Program: Option 4

The Recreational Use Public Health Advisory Guidelines document explains that a monitoring program based on toxins rather than cell counts will likely result in fewer and shorter duration

public health advisories but may have higher sampling costs. Toxin-based monitoring programs also provide the most accurate information in terms of protecting public health, because toxins pose actual risk rather than the potential risk posed by the presence of cyanobacterial cells.

If Option 4 is chosen, an advisory will not be issued unless toxin testing shows levels above the recreational use values shown in Table 1. In the interim, the public should be notified that a bloom has been identified and toxin testing is underway or ongoing. The public should be advised to avoid water that is foamy, scummy, thick like paint, pea-green, blue-green or brownish red. Contact OHA for signage options or templates.

The recommended approach to maintaining a toxin-based monitoring program is as follows:

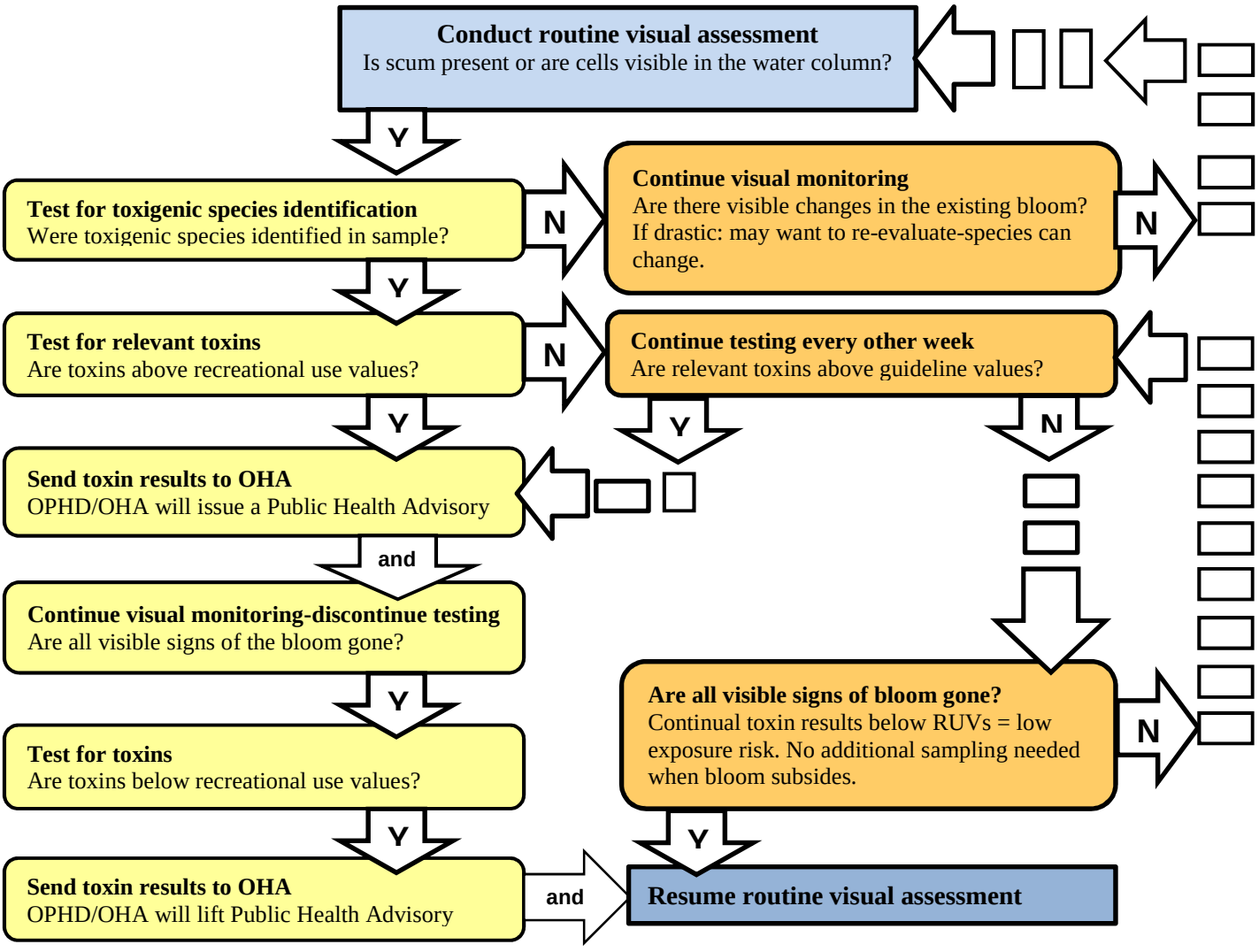

Table 1. Recreational use values (RUVs) for cyanotoxins in Oregon's waters (µg/L)

RUVs:	Microcystin	Anatoxin-a	Saxitoxin	Cylindrospermopsin
	4	8*	4*	8

***** The RUVs for anatoxin-a and saxitoxin have been updated from previous values based on research performed by the EPA for microcystin and cylindrospermopsin. Appendix B of OHA's Recreational Use *Public Health Advisory Guidelines*discussesthe rationale behind the change.

Sampling frequency for this option is every other week if toxin-producing species are present but the toxin levels are below guideline values. No recreational use advisory would be issued as long as toxin levels stay below the RUVs. When using Option 4, OHA requests that sampling results be available within 3 business days after the lab receives the sample. This helps us communicate with the public in a timely manner and demonstrates that action is being taken to protect the public's health. Before hiring a lab to analyze a sample, we suggest contacting them to discuss turnaround time to ensure that this 3-day standard can be met. For OHA purposes, electronic versions with official results are acceptable.

If levels are found to be above guideline values, then an advisory is issued, advisory signage is posted, and sampling can be suspended until the bloom is gone. To lift the advisory, samples must show that toxin levels are below guideline values.

NOTE: OHA is interested in copies of all toxin and cell speciation data for tracking purposes, even if the levels found are below for issuing an advisory. Providing these results is not required for participation in OHA's cyanoHABs program. However, it helps OHA build a statewide system for monitoring trends in cyanoHABs in Oregon waters. OHA takes no action on any data submitted unless results are above the recreational use values.

Laboratories

The number of laboratories available for species identification, cell counts, and toxin analysis is limited. Laboratories providing cyanobacterial analysis are listed in Appendix C. The accepted method for determining cell counts is Standard Methods Section 10200E and F (also called "SM10200"). Some water body managers prefer cell count data as resources are limited and cell count information is affordable.

Toxin analysis is required to lift all recreational use health advisories for cyanoHABs. Cyanotoxin tests currently available through commercial laboratories use a variety of comparable methods. Tests can be costly, depending on the information requested, and the method and equipment used. OHA recommends contacting your lab for current costs, and for instructions on preserving and shipping the sample.

As a rule of thumb, the ELISA method is least expensive for determining levels of microcystin, saxitoxin, and cylindrospermopsin in the bloom. ELISA methods are not currently available for anatoxin-a, however, Abraxis has introduced a micro-titer plate format (96T), receptor-binding assay (RBA) kit for Anatoxin-a. This kit provides two protocols. The EZ protocol requires no sample preparation and has a range of 5 to 500 ppb. If a lower limit of detection is required, the enhanced sensitivity (ES) SPE sample protocol may be performed. The kit provides a real-time, economical,

accurate and sensitive alternative for both research and monitoring**.** OHA does not accept field ready test kits or dip stick tests for any cyanotoxins as a basis for lifting an advisory. However, these kits may be useful for monitoring the progress of a bloom.

When testing for cyanotoxins, ensure the lab uses a method with a detection level below the RUVs shown in Table 1 (page 8).

It is important to test for toxins that may be produced by the dominant species of cyanobacteria in a bloom. To do so, it is necessary to identify the dominant species present (with or without enumeration). Consult Table 2 to determine the associated toxins.

Table 2. Toxigenic cyanobacteria (data derived from evidence of toxin production presented in Sivonen and Jones, 1999; Carey et al., 2007; Funari and Testai, 2008 and Voloshko, 2008)[1]

	Hepatotoxins			Neurotoxins	
	Microcystin	Nodularin	Cylindro-spermopsin	Anatoxin-a	Saxitoxin
Anabaenopsis	+				
Aphanizomenon (Except A. flos-aquae)			+	+	+
Arthrospira	+				
Cyanobium	+				
Cylindrospermopsis			+		+
Dolichospermum (formerly Anabaena)	+		+	+	+
Gloeotrichia	+				
Hapalosiphon	+				
Limnothrix	+				
Lyngba					+
Microcystis	+			+	
Nodularia		+			
Nostoc	+				
Oscillatoria	+			+	
Phormidium	+			+	
Planktothrix	+			+	+
Raphidiopsis			+	+	
Schizothrix					
Synechocystis	+				
Umezakia			+		

[1]*Taxonomy for many types of cyanobacteria is currently being revised. This guidance reflects taxonomy as of January 2017.*
Table 2 is at the genus level, not the species level. Not all species of a given genus produce all toxins listed for that genus. Once the species involved in a specific bloom have been identified, it is recommended that waterbody mangers contact OHA to determine exactly which toxins could be involved.

For the purpose of public health advisories, and until research has been completed to determine otherwise, Aphanizomenon flos-aquae (AFA) is excluded from calculation of combined cell counts of toxigenic species. Refer to Recreational Use Public Health Advisory Guidelines for more information.

Shipment of samples

Collection volume, storing methods, holding times and shipping guidance is provided in Appendix B. However, OHA recommends that you contact the lab performing the sample analysis prior to sample collection, to discuss these details with the laboratory that will be performing the analyses. The lab can give you all the information needed, and in many cases will provide the cooler and other materials for shipping.

Samples should be shipped the same day as collected. Ship samples in a cooler or a cardboard box lined with plastic bags (to prevent leakage) and newspaper or other material for insulation.

For toxin samples, include ice bags, ice packs, or other cooling products (e.g., blue ice) to ensure the samples stay cold. If shipping more than one sample for algae identification, enumeration and/or toxin analysis to the same lab, you may package them in the same cooler to save on shipping costs.

Program contact information

Email: habhealth@state.or.us
Phone: (971) 673-0440
Website: www.healthoregon.org/hab

Appendix A: Field assessment sheet

Please gather the following information for each location assessed, if applicable.

General Information

Assessment date (mm/dd/yyyy) ___/___/_____ Time _____AM / PM*(circle one)*

Name of waterbody _____ County _____

Assessment location description/number _____

Assessment Location Latitude_____ Longitude _____

Visual Assessment

Water clarity *(circle all that apply):* Clear Cloudy Hazy Opaque Don't know

Water color *(circle all that apply):* Green Blue-green Brown Rust Milky White
Red Pink Clear Don't know

Visible bloom *(circle one)*: Yes No Don't know Visible scum *(circle one):* Yes No Don't know

Reason for assessment: Monitoring/Sampling Fish kill Health event response Other _____

Recreational Area Assessments

Total number of people in the recreational area _____Of those, number of people in the water _____

Total number of boats in use _____ Number of people waterskiing/boarding _____

Total number of dogs in the recreational area_____ Of those, number of dogs in the water _____

Other Observations and Comments *(e.g., sketch bloom location in respect to recreational areas)*

Sample Collector Information

Name _____ Position _____

Phone Number _____ Agency/Organization _____

Appendix B: Step-by-step sample collection instructions

(Contact the laboratory prior to sampling to confirm collection and preservation requirements)

Sampling Equipment Checklist (confirm with laboratory)

- ☐ *Field Assessment Sheet*
- ☐ *Arm-length Disposable Waterproof Gloves*
- ☐ *Hip Waders*
- ☐ *Eye Protection/Safety Glasses*
- ☐ *Waterproof Permanent Marker/Pen*
- ☐ *4 liter stainless steel bucket (if composite sampling)*
- ☐ *Cooler or insulated shipping box*
- ☐ *Ice Pack(s)*
- ☐ *GPS Unit (recommended)*
- ☐ *500 mL HDPE Plastic Sample Bottles*
- ☐ *Lugol's Preservative Solution*
- ☐ *Long-handled Stainless Steel Stirring Spoon (if composite sampling)*

Sampling Instructions

Sample Event Preparation

1. Prepare Field Assessment Sheets (Appendix A). Use one sheet per sample location to describe ambient conditions. This will help to interpret results, as well as provide sample collection contact information. Use pencil or waterproof permanent marker or pen when completing the sheet.
2. Label each bottle with the sample location and either "Cell & Species" or "Toxins" as needed. Use pencil or waterproof permanent marker or pen when completing the labels.

Collecting Samples- *Wear disposable gloves and any other necessary personal protective equipment.*

1. Select a sampling area that represents a distinct cyanoHAB and/or distinct risk of exposure (for example, a swim beach). Within that sampling area, select the location (or three locations, if composit sampling) with the highest concentration of cyanobacteria.
2. If taking the sample from the shoreline, wade or boat slowly to the sampling location. If wading or boating, approach on the downwind side and avoid agitating the water or sediment.
3. Disturb the water at the sample location for approximately ten seconds to simulate conditions created by a swimmer or wader coming into contact with the cyanobacteria.
4. Remove the cap from the sample bottle, tilt the bottle approximately 45 degrees and allow the bottle to fill as you submerge it 3 to 6 inches below the surface.
5. Replace the cap and remove any cyanobacteria adhered to the outside of the bottle. Turn the bottle end-over-end three or four times to mix.
6. If creating a composite sample:
   a. Because cyanotoxins are organic compounds, sampling equipment used to collect and combine samples should be made of fluorocarbon polymers (such as Teflon®); metals (such as stainless steel); or glass.
   b. Rinse the bucket with fresh water or with lake water relatively free from cyanobacteria.

c. Pour the three sample bottles representing each sampleinto the bucket. (*Remember, these are samples taken at a particular point across time, at many depths in a single location, or at a given depth in multiple locations).*
d. Use a long-handled stainless steel stirring spoon to thoroughly mix the three samples. Do not stir vigorously as this type of mixing may rupture (lyse) the cyanobacteria cells. If lysing occurs, cell enumeration becomes very difficult to perform with accuracy. This is less of an issue for toxin samples since cyanobacteria cells in the sample will be lysed at a later stage of processing.
e. Pour into a rinsed sampling bottle. Replace the cap and remove any cyanobacteria adhered to the outside of the bottle. Turn the bottle end-over-end three or four times to mix.

Sample preservation – (*confirm with laboratory)*

1. Cell Enumeration & Species Identification Samples: Work with *preservatives in well ventilated areas like outside or under a fume hood. Wear gloves and safety glasses.*
    a. Remove the cap and pour off enough of the sample to leave one inch air space for mixing the Lugol's solution.
    b. Using a pipette, calibrated dropper or medicine cup, measure the correct amount of preservative and add to the sample bottle. Usually 5 mL Lugol's solution (or another preserving agent recommended by the lab).
    c. Close the cap tightly and turn the bottle end-over-end three or four times to mix.

Shipping Instructions

A. Cell enumeration and species identification: Place samples on ice in a cooler or insulated well packed box and arrange overnight shipping of the samples to the laboratory.

B. Toxin Samples:

    Place bottle(s) on ice as soon as possible in a cooler (preferable) or insulated box and arrange overnight shipping of the sample to the laboratory.

Samples must arrive at the lab cold. If shipping must occur before a weekend, ensure enough ice or cooler bags are included so that the temperature of the samples is not compromised over the weekend. Analysis can still be completed, but the results will not be as accurate.

Appendix C: Available laboratories for cyanobacteria analysis

When processing water samples for toxins as part of a toxin-based monitoring program, OHA recommends sampling data be available within 3 business days after receipt of a sample. This helps OHA and the waterbody managers to meet the need for timely communication with the public and demonstrates that action is being taken to protect public health. For OHA purposes, electronic versions with official results will suffice.

Aquatic Analysts
(Identification and enumeration)
43 Telegraph Lane
Friday Harbor, WA 98250

Attn: Jim Sweet
Phone: (503) 869-5032
jwsweet@aol.com

Aquatic Services
(Consulting, identification and enumeration)
42184 Tweedle Lane
Seaside, OR 97138

Attn: Wayne W. Carmichael, PhD
Phone: (937) 620-4603or
(503) 755-0711
wayne.carmichael@wright.edu

Beagle Bioproducts, Inc.
(Toxin analysis)
959 Schrock Road
Columbus, OH 43229

Attn: Stephanie A. Smith, PhD
Phone: (614) 519-0154 (cell)
www.beaglebioproducts.com
stephanie.smith@beaglebioproducts.com

Bend Genetics LLC
(Toxin analysis)
87 Scripps Dr. Ste 301
Sacramento, CA, 95825

Attn: Customer Service
Phone: (916) 550-1048
Fax: (916) 515-8093
customer_service@bendgenetics.com

CAHFS Toxicology Laboratory
(Toxin analysis)
University of California
School of Veterinary Medicine
West Health Sciences Drive
Davis, CA 95616

Attn: Birgit Puschner
Phone: (530) 752-6322
Fax: (530) 752-3361
bpuschner@ucdavis.edu

CH2M HILL Applied Science Laboratory
(Toxin analysis - Microcystin)
1000 NE Circle Blvd., Suite 10350
Corvallis, OR 97330

Attn: Lab Customer Service Support
Phone: (541) 768-3120
Fax: (541) 766-2852
asl@ch2m.com

EcoAnalysts, Inc.
(Toxin analysis, identification and enumeration)
1420 S. Blaine St., Suite 14
Moscow, ID 83843

Phone: (208) 882-2588
Fax: (208) 883-4288
eco@ecoanalysts.com

GreenWater Laboratories/Cyano Lab
(Toxin analysis, identification and enumeration)
205 Zeagler Drive, Suite 302
Palatka, Florida 32177

Phone: (386) 328-0882
Fax: (386) 328-9646
markaubel@greenwaterlab.com

King County Environmental Laboratory
(Toxin analysis, identification and enumeration)
322 West Ewing Street
Seattle WA 98119

Attn: Fran Sweeney
Phone: (206) 684-2358
Francis.sweeney@kingcounty.gov

LSSU Environmental Analysis Laboratory
(Identification, enumeration, toxin testing)
Lake Superior State University
650 W. Easterday Avenue, Sault Ste.
Marie, MI 49783

Attn: Ben Southwell
phone: (906) 635-2076
bsouthwell@lssu.edu

MWH Laboratories
(Toxin analysis, identification and enumeration)
750 Royal Oaks Dr., Ste. 100
Monrovia, CA 91016

Attn: Dr. Andrew Eaton
Phone: 1 (800) 566-5227

PhycoTech
(Identification and enumeration)
620 Broad Street, Suite 100
St. Joseph, MI 49085

Phone: (269) 983-3654
Fax: (866) 728-5579
info@phycotech.com

WATER Environmental Services, Inc.
(Identification and enumeration)
9515 Windsong Loop NE
Bainbridge Island, WA 98110

Attn: Maribeth Gibbons, Pres.
Phone: (206) 842-9382
mvg.water@gmail.com

Water Management Laboratories
(Toxin analysis, identification and enumeration)
1515 80th St. E
Tacoma, WA 98404

Attn: Diane DuMond
Phone: (253) 531-3121