



NJ Department of Environmental Protection
Division of Water Monitoring and Standards
Bureau of Freshwater & Biological Monitoring

Cyanobacterial Harmful Algal Bloom (HABs) Freshwater Recreational Response Strategy



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The New Jersey Department of Environmental Protection (DEP) wishes to acknowledge the input of the members of the interagency Harmful Algal Bloom (HAB) Workgroup in the development of this Strategy (a listing of the members can be found in Appendix A). Workgroup members represent the following agencies/programs: DEP – Division of Water Monitoring and Standards/Director’s Office and Bureau of Freshwater & Biological Monitoring, Water Resource Management, Division of Science, Research and Environmental Health, the Division of Water Supply and Geoscience, State Park Service, Division of Fish & Wildlife, and the Office of Quality Assurance; New Jersey Department of Health (DOH) – Division of Epidemiology, Environmental and Occupational Health/Consumer, Environmental and Occupational Health Service, and Communicable Disease Service; New Jersey Department of Agriculture - Division of Animal Health.

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Acronym List

AC - Assistant Commissioner

ADDA - cyclic heptapeptide structure of the general composition cyclo(-D-Ala-L-X-D-erythro- β -methylisoAsp-L-Y-Adda-D-iso-Glu-N-methyldehydroAla), where ADDA is the unusual C20 aa 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and X and Y are variable L-aa.

BFBM - DEP Bureau of Freshwater and Biological Monitoring

C & E - DEP Compliance and Enforcement

CDC - Center for Disease Control, United States Department of Health and Human Services

CDS - DOH Communicable Disease Service

CEHA - DEP County Environmental Health Act program

CEOHS - DOH Consumer, Environmental and Occupational Health Service

DEP – New Jersey Department of Environmental Protection

DOH – New Jersey Department of Health

DSREH - DEP Division of Science, Research and Environmental Health

DWMS - DEP Division of Water Monitoring and Standards

DWSG -DEP Division of Water Supply and Geoscience

EEOH - DOH Environmental and Occupational Health

ELISA - Enzyme-Linked Immuno-Sorbent Assay

HAB - Harmful Algal Bloom

LC-ESI/MS/MS - Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry

LC/MS/MS - Liquid Chromatography/Tandem Mass Spectrometry

NLA - National Lakes Assessment, USEPA

OHHABS - CDC One Health Harmful Algal Bloom System

qPCR - quantitative polymerase chain reaction

UCMR - Unregulated Contaminant Monitoring Rule, USEPA

USEPA - United State Environmental Protection Agency

USGS - United States Geological Survey

WHO - World Health Organization

WMA - Wildlife Management Area

WRM - DEP Water Resource Management

1. PURPOSE AND SCOPE

The purpose of this New Jersey Cyanobacterial Harmful Algal Bloom (HAB)* Response Strategy is to provide a unified statewide approach to responding to cyanobacterial HABs in freshwater recreational waters and sources of drinking water, and to protect the public from risks associated with exposure to cyanobacteria and related toxins. Although the primary focus is on the protection of human health, this Strategy provides some information and recommendations regarding prevention of exposure to domestic animals, wildlife, and livestock as well. The Response Strategy is designed to identify:

- Entities responsible for response and actions
- Recreational risk thresholds
- Acceptable parameters and methods for assessing risk
- Appropriate monitoring and analysis for toxins
- Recommended Advisories and other appropriate communication mechanisms

The scope of the Response Strategy is for lakes, rivers, and streams with potential public access, recreational use, bathing beaches (including licensed beaches), and sources of drinking water. These waterbodies may be owned or operated by state, county, municipal, federal or private entities. As such, coordination of the investigation and response activities will vary depending on ownership.

Direct drinking water concerns are addressed in DEP's Division of Water Supply and Geoscience (DWSG) Emergency Response Plan, which focuses on prevention and treatment of drinking water contamination, and applies to cyanobacterial HABs and toxins. DWSG will coordinate action with appropriate water supply authorities in the event of a drinking water contamination. DWSG requires water utilities that are at risk for a HAB to plan for such events as part of their Emergency Response Plan. In addition, DWSG is working with these utilities to develop management plans based on the 2015 USEPA "Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water". For more information on drinking water see the DWSG website:

<http://www.nj.gov/dep/watersupply/>.

A. Agency Responsibilities

An interagency HAB Workgroup was formed, consisting of representatives from the New Jersey Department of Environmental Protection (DEP), the New Jersey Department of Health (DOH), and the New Jersey Department of Agriculture to discuss and collaborate on issues including: Response Strategy development, monitoring and analysis advancements, risk thresholds, advisories, research and communication. Following development of this Response Strategy, the Workgroup will continue to meet periodically to enhance the Strategy based on New Jersey's experience with cyanobacterial HABs, available information and science, as well as available federal guidance. Appendix A contains a list of the members of the Workgroup and contact information. Appendix B provides the local/county Health Department emergency contact information for this Strategy.

* For this Strategy document, a HAB refers to a cyanobacterial Harmful Algal Bloom.

The following are the responsibilities of each state agency tasked with contributing to this Strategy. Any agency that initiates an investigation of a potential Harmful Algal Bloom in freshwater recreational waters is encouraged to complete and submit the Bureau of Freshwater and Biological Monitoring's (BFBM's) "Potential Harmful Algal Bloom Report – Freshwater" (refer to Appendix D).

NJ Department of Environmental Protection (DEP)

Division of Water Monitoring and Standards/ Director's Office and Bureau of Freshwater and Biological Monitoring (DWMS/BFBM)

- Develop monitoring and analysis capacity for cyanobacteria/cyanotoxins.
- Perform surveillance for freshwater HABs while staff are conducting other field sampling, monitoring, and reconnaissance work on lakes, rivers and streams.
- Oversee overall HAB information dissemination and outreach including production and maintenance of general HAB information and fact sheets on DWMS homepage and DWMS/BFBM websites.
- Develop and maintain HAB reporting form. Collect and review reports following submissions, and determine who should be contacted for follow-up.
- Monitor and analyze blooms. Depending on waterbody jurisdiction and use, may include direct monitoring and analysis by DWMS/BFBM and/or coordination and guidance for monitoring and analysis of blooms.
- Coordinate implementation of HAB Strategy with other NJ State and Federal agencies.
- Coordinate investigation and response with appropriate partners. Internal partners of NJDEP include the program areas of Fish and Wildlife, Parks and Forestry, Compliance & Enforcement, Water Supply & Geoscience, and external partners such as the county and/or local health departments.
- Coordinate with NJ Department of Health information dissemination and outreach to local health departments and the public regarding the effects of HABs.
- During Business Hour notifications, the NJDEP's Communication Center documents potential HAB Hotline Notifications as "algal blooms" and forwards the incidents to the BFBM
- During Business Hours, upon notification of a potential HAB incident (Algal Bloom), NJDEP's BFBM will serve as the lead to investigate and coordinate responses consistent with Section 4 of this document, as applicable to the incident. Primary activities include completing the initial incident report, performing field activities involving visual assessment and field screening (cyanobacteria and toxin presence), conducting laboratory analysis, and coordinating appropriate response activities, such as the issuance of advisories.

DEP State Park Service

- Visually monitor State Park lakes for HAB development. BFBM will provide necessary materials, such as toxin test strips, as needed.
- Contact DWMS/BFBM when blooms are sighted at licensed bathing beaches or in other contact-recreational areas for sample collection and analysis
- Post advisories at State Park lakes using guidelines in this document (Section 5). Also, posts on Facebook page and website.
- After initial response and issuance of advisory, it is the responsibility of Parks and Forestry to communicate status to DWMS/BFBM throughout the HAB event, until the advisory is ultimately

lifted. Provide outreach to the public about HABs.

- Coordinate with lake management contractors to manage State Park lakes for the prevention of HABs
- Assist in sample collection support as situation warrants, such as an event that exceeds the resource capability of DWMS/BFBM. Sample collection for HABs follows the method for Phytoplankton Sampling, Chapter 6.10.1 of the NJDEP Field Sampling Procedures Manual, August 2005, <http://www.state.nj.us/dep/srp/guidance/fspm/>.

DEP Division of Science, Research and Environmental Health (DSREH)

- Provide cyanoHAB scientific support and summarize human health exposure and impacts
- Provide technical consultation regarding bloom response
- Establish and/ or revise guidelines/thresholds for cyanobacteria and related toxins for recreational risk and drinking source water risk
- Define acceptable parameters and methods for assessing risk
- With DWMS/BFBM, research new developments in HAB monitoring, analysis, prediction, treatment and impacts

DEP Division of Water Supply and Geoscience (DWSG)

- Maintains Emergency Response Plan, which focuses on prevention and treatment of drinking water contamination, and applies to cyanobacterial HABs and toxins.
- Coordinate with DWMS/BFBM regarding source water HABs, including reservoirs used for both drinking water and recreational activities
- Coordinate appropriate response to HAB events with water purveyors, including but not limited to:
 - Identification of and/or approval of use of alternate supply where feasible.
 - Timely and appropriate sampling, reporting, and communication of results with relevant agencies.
 - Appropriate alteration of treatment techniques.
 - Provision of technical assistance as needed.
 - Public notification, including appropriate drinking water advisories in multiple languages as needed.
- Interact with and report to appropriate emergency response officials as set forth in an incident command structure.
- Perform after-action evaluation to improve future response and to make management and prevention recommendations.

DEP Fish and Wildlife

- Visually monitor lakes and streams during scheduled field sampling activities for HAB development. Contact DWMS/BFBM when blooms are sighted in contact-recreational areas for sample collection and analysis.
- Post advisory signs at Wildlife Management Area (WMA) lakes. (Refer to Section 5 for guidance).
- After initial response and issuance of advisory, it is the responsibility of Fish and Wildlife to communicate status to DWMS throughout the HAB event, until the advisory is ultimately lifted.
- Provide outreach to the public about HABs.
- When necessary, NJDEP Fish and Wildlife will submit liver tissue samples from fish and wildlife cases with suspected mortality from HABs to Pennsylvania Animal Diagnostic lab (PADL) for

confirmation of tissue toxins

DEP Compliance and Enforcement/ Division of Water and Land Use Enforcement

- During After Hour notifications, the NJDEP's Communication Center documents potential HAB Hotline Notifications as "algal blooms" and forwards the incidents to the appropriate regional office of Water and Land Use Enforcement.
- After-hours, upon notification from the Communication Center, NJDEP's Compliance & Enforcement (C&E) program will serve as lead to initiate an investigation for HAB incidents using BFBM's "Potential Harmful Algal Bloom Report – Freshwater" (refer to Appendix D).
- Until the incident is referred to BFBM, C&E also functions to promote initial coordination by making the following notifications as applicable, for incidents potentially impacting a drinking water source, or licensed freshwater bathing beaches:
 - ✓ Contact DWSG if the potential HAB occurrence is reported in a drinking water source.
 - ✓ Contact the State Park superintendent if the potential HAB occurrence involves a licensed bathing beach at a State Park (13 State Parks with freshwater licensed bathing beaches)
 - ✓ Contact the local/county Health Department and DOH if the potential HAB occurrence affects non-state owned bathing beaches. Refer to contact list in Appendix B.
 - ✓ Contact the appropriate regional office of Fish & Wildlife if the incident adversely impacts wildlife (i.e. fish kill).
 - ✓ Contact DWMS - BFBM during first available business hours for continued investigation and response coordination.

After-hour notifications by C&E can be accomplished using DEP's Emergency Response Coordinators list for each program area (DEP internal use only).
- Assistance from regional offices in sample collection support as situation warrants, such as an event that exceeds the resource capability of DWMS/BFBM. Sample collection for HABs follows the method for Phytoplankton Sampling, Chapter 6.10.1 of the NJDEP Field Sampling Procedures Manual, August 2005, <http://www.state.nj.us/dep/srp/guidance/fspm/>. Safety training for sample collection will be provided by DWMS/BFBM if necessary.

DEP Emergency Management Program

- Maintain the functionality of the DEP Hotline/Communication Center to gather and share incident reports involving a potential HAB occurrence in freshwater.
- Assist with incident management as needed.

New Jersey Department of Health (DOH)

Division of Epidemiology, Environmental and Occupational Health/Consumer, Environmental and Occupational Health Service & Communicable Disease Service

- Advise and make appropriate recommendations regarding licensed, recreational bathing facilities, including NJ State Park bathing facilities
- Maintain and provide to NJDEP (for response purposes) a list of licensed freshwater bathing beaches with local health department emergency contact information.
- Offer technical assistance and consult with NJDEP regarding any HAB health related concerns in freshwaters regardless of bathing designation.
- Coordinate with and inform local health departments regarding appropriate response and advisories - Local health authorities license and inspect within their jurisdictions public recreational bathing facilities.
- Confirm advisories and/ or other actions have taken place
- Review illness reports and determine if they are related to cyanotoxins
- Determine advisory thresholds in consultation with NJDEP
- Provide information to the public regarding HAB awareness.
- Provide outreach to the public about the health effects of HABs, in conjunction with NJDEP

New Jersey Department of Agriculture

Division of Animal Health/ New Jersey Animal Emergency Response

- Receive and review notifications by NJDEP of any HAB occurrence that may affect livestock.
- Notify DWMS/BFBM of any reports received by Dept. of Agriculture.
- Notify and issue advisories to livestock owners as appropriate to protect livestock health.
- After initial response and issuing of an advisory, communicate status to livestock owners until the advisory is ultimately lifted.

2. BACKGROUND

A. Cyanobacteria

Cyanobacteria are a type of bacteria capable of photosynthesis. Although they are not true algae, they are often referred to as “blue-green algae”. Cyanobacteria frequently impart off-tastes and odors to the water in which they grow. Some species can produce toxins (known as Cyanotoxins) that can be harmful to the health of humans and other animals. Although problems related to cyanobacteria most often occur in freshwaters (lakes and streams), cyanobacteria can also be found in marine waters.

A cyanobacterial Harmful Algal Bloom (HAB) is the name given to the excessive growth, or “bloom”, of cyanobacteria, some of which can produce one or more types of potentially harmful toxins. HABs can occur under suitable environmental conditions of light, temperature, nutrients, and calm water. These “blooms” often result in a thick coating or “mat” on the surface of a waterbody, often in late-summer or early fall. A general overview fact sheet about Cyanobacterial Harmful Algal Blooms (HABs) as well as a technical fact sheet related to recreational exposure and health effects are available at: <http://www.state.nj.us/dep/wms/bfbm/>. Both fact sheets are also located in Appendix C.

B. Cyanobacterial Blooms

Cyanobacterial blooms may vary in species community composition, residence time, toxins they produce, and toxicity and risk to human health, pets, livestock and wildlife. The distribution and concentration of blooms may be affected by weather and lake conditions such as rain, wind, and currents. Distributions can be lake or waterbody-wide or localized near the shoreline, shallows, or areas affected by flows or the influx of nutrients.

Cyanobacteria may maintain a position at a particular depth, or may be found throughout the water column where light penetrates (e.g. *Planktothrix*, *Cylindospermopsis*). Cyanobacteria may migrate vertically to different locations in the photic zone (where light penetrates) throughout the day. Surface accumulations (scum) may develop when cyanobacteria float to the surface during calm, sunny weather and may dissipate within hours as conditions change.

Entire cyanobacteria populations may accumulate at 1 or 2 cm below the water surface. Surface accumulations of cyanobacteria may concentrate further when blown by wind to leeward areas like bays, inlets, or near-shore areas (with the direction of the wind). Dense accumulations may extend from the surface to depths more than 1 m.²



3. HUMAN HEALTH RECREATIONAL RISK THRESHOLDS

This section is intended to provide guidelines for NJ entities responsible for responding to CyanoHABs regarding their potential risk to human health in recreational freshwaters.

A. Human Health Impacts from Exposure to Cyanobacteria and Toxins (Cyanotoxins)

The most common exposures to cyanobacteria and their toxins are believed to occur during recreational activities by mouth, skin, and inhalation routes. Oral exposure may occur from accidental or deliberate ingestion of water. Dermal exposure may occur by direct contact of exposed parts of the body during recreational activity in water containing cyanobacteria. Inhalation may occur through the inhalation of contaminated aerosols while recreating.

Adverse health effects from recreational exposure to cyanobacterial cells and cyanotoxins can cause effects ranging from a mild skin rash to serious illness or death. Acute illnesses caused by exposure to cyanotoxins have been reported.

Allergic-like reactions (e.g., rhinitis, asthma, eczema, and conjunctivitis) to flu-like reactions (e.g., skin rashes, gastroenteritis, and respiratory irritation) can occur through recreational exposure. Allergic or irritative skin reactions of varying severity have been reported from recreational exposures where the presence of freshwater cyanobacteria, such as *Anabaena* (Figure 2), *Aphanizomenon*, *Nodularia*, and *Oscillatoria* endotoxins have been confirmed. Skin and eye irritation, from exposure during swimming, have been related to the blue-green pigment of the Cyanotoxins (phycocyanin) and dermal toxins produced by the *Lyngbya* and *Planktothrix* species.

In addition, microcystins and anatoxin-a have been linked to gastrointestinal illness, liver disease, neurological effects, skin reactions, and possible cancer in humans. Experimental studies have demonstrated the tumor promotion activity of microcystins and nodularin. (USEPA's HABs website: <https://www.epa.gov/nutrient-policy-data/health-and-ecological-effects#how1>)

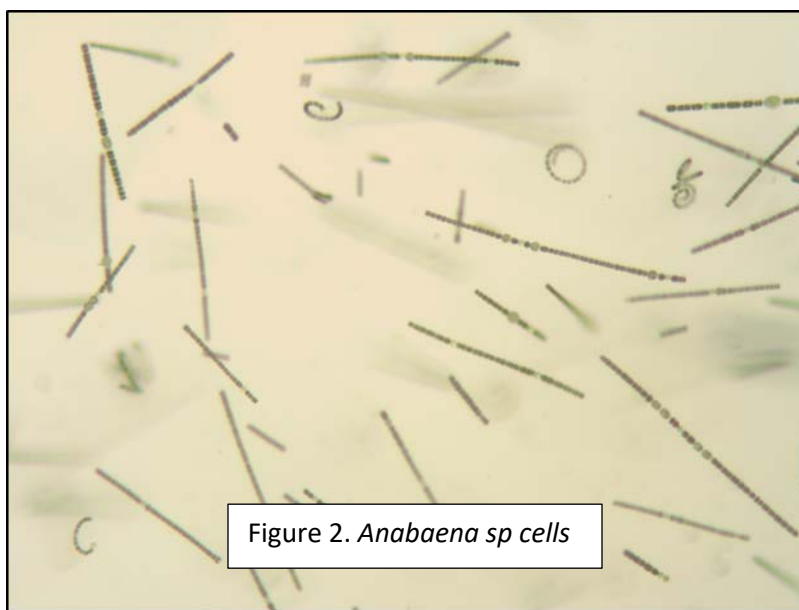


Figure 2. *Anabaena sp* cells

Table 1 lists the primary Cyanotoxins as well as their associated human health effects.

Table 1. Primary Cyanotoxins and their Associated Human Health Effects (USEPA's HABs website : <https://www.epa.gov/nutrient-policy-data/cyanohabs>)

Cyanotoxin	Acute Health Effects in Humans	Most Common Cyanobacteria Producing the Toxin
Microcystin-LR	Abdominal Pain, Headache, Sore Throat, Vomiting and Nausea, Dry Cough, Diarrhea, Blistering around the mouth, Pneumonia, and liver toxin.	<i>Microcystis</i> , <i>Anabaena</i> , <i>Nodularia</i> , <i>Planktothrix</i> , <i>Fischerella</i> , <i>Nostoc</i> , <i>Oscillatoria</i> , and <i>Gloeotrichia</i>
Cylindrospermopsin	Fever, Headache, Vomiting, Bloody Diarrhea, Liver Inflammation, and Kidney Damage	<i>Cylindrospermopsis raciborskii</i> , <i>Aphanizomenon flos-aquae</i> , <i>Aphanizomenon gracile</i> , <i>Aphanizomenon ovalisporum</i> , <i>Umezakia natans</i> , <i>Anabaena bergii</i> , <i>Anabaena lapponica</i> , <i>Anabaena planctonica</i> , <i>Lyngbya wollei</i> , <i>Raphidiopsis curvata</i> , and <i>Raphidiopsis mediterranea</i>
Anatoxin-a group	Tingling, Burning, Numbness, Drowsiness, Incoherent Speech, Salivation, Respiratory Paralysis Leading to Death	<i>Chrysosporum</i> (<i>Aphanizomenon</i>) <i>ovalisporum</i> , <i>Cuspidothrix</i> , <i>Cylindrospermopsis</i> , <i>Cylindrospermum</i> , <i>Dolichospermum</i> , <i>Microcystis</i> , <i>Oscillatoria</i> , <i>Planktothrix</i> , <i>Phormidium</i> , <i>Anabaena flos-aquae</i> , <i>A. lemmermannii</i> , <i>Raphidiopsis mediterranea</i> (strain of <i>Cylindrospermopsis raciborskii</i>), <i>Tychonema</i> and <i>Woronichinia</i>

How are people or animals that have been exposed to cyanobacteria and cyanotoxins treated?

The Center for Disease Control (CDC) states that if you or your pet comes in contact with a cyanobacteria bloom, you should wash yourself and your pet thoroughly with fresh water. If you or your pet swallow water from where there is a harmful algae bloom, call your doctor, a Poison Center, or a veterinarian. Call a veterinarian if your animal shows any of the following symptoms of cyanobacteria poisoning: loss of appetite, loss of energy, vomiting, stumbling and falling, foaming at the mouth, diarrhea, convulsions, excessive drooling, tremors and seizures, or any other unexplained sickness after being in contact with water. For more information see the CDC website:

<http://www.cdc.gov/habs/materials/factsheets.html>.

B. Cyanobacteria and Cyanotoxin Risk Thresholds for Recreational Waters

EPA has developed draft guidance values for one or more cyanotoxins <https://www.epa.gov/wqc/microbial-pathogenrecreational-water-quality-criteria#swimming>, while a number of states, as well as the World Health Organization (WHO), have derived their own “action levels” or health advisory guidelines based on either cyanobacteria cell counts, or concentrations of a few of the more toxic, commonly-occurring cyanotoxins (Appendix H). The WHO report is available at: http://www.who.int/water_sanitation_health/publications/srwe1/en/.

Below are threshold levels developed specifically for New Jersey by the DEP Division of Science, Research and Environmental Health (DSREH) for three of the most commonly observed cyanobacterial toxins: microcystin-LR, cylindrospermopsin, and anatoxin-a.

DWMS/BFBM has developed the laboratory capability to measure levels of these three toxins within the specified threshold limits.

C. Recommended Action Level and Health Advisory Guidance Levels

DEP/DSREH has developed recommended levels intended to be protective of a range of exposures and are probably highly conservative (i.e., protective) for the exposures most likely to occur. The document “NJDEP Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin –a” is located in Appendix I. The uncertainties in the risk estimates, as well as the inherent uncertainty in the temporal variability of the toxins in any given waterbody, should be considered when providing advice to the public regarding recreation in affected waterbodies.

- **Action level based on cyanobacterial cell count**

Low concentrations of cyanobacteria may cause allergenic and/or irritative effects to a portion of an exposed population. These effects are caused by endotoxins (mainly the lipopolysaccharide component of the cyanobacterial cell wall) rather than cyanotoxins. Therefore, county or local authorities should post advisories for any freshwater lake or pond in which cyanobacterial cell counts reach a level of concern. If the cyanobacterial cell count equals or exceeds 20,000 cells/ml in an area where primary recreational contact is likely to occur, county or local authorities should use this as the basis of posting advisory signs. This recommendation is based on WHO(2003a) guidance described in detail in the full DSREH document located in Appendix I.

- **Health advisory guidance levels for individual cyanotoxins**

DEP/ DSREH recommends the following guidance values for recreational exposure to individual cyanotoxins. The basis, including derivation of reference doses and explanation of exposure assumptions, is provided in Appendix I.

- Microcystins (as total including –LR and other detectable congeners): 3 µg/L
- Cylindrospermopsin: 8 µg/L
- Anatoxin-a: 27 µg/L

4. RESPONSE TO HABS IN RECREATIONAL WATERS

A. Initial Report

A HAB often looks like a layer of bright bluish-green or white paint on the water surface. Other evidence of a potential cyanobacterial HAB could be discolored or pea-green colored water, parallel streaks, or green dots/globs in the water. It is important to note that some blooms are due to common green algae and not cyanobacteria and, when present, cyanobacteria do not always produce cyanotoxins.

If you observe what you think might be a HAB in a pond, lake, or stream, call the DEP Hotline (1-877-WARNDEP) to report it.

DWMS/BFBM has developed a HAB-related reporting form (See Figure 3 below and Appendix D) available at: www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. This report form is intended for both public and private waterbodies and is intended to be completed in addition to the DEP Hotline report (see Figure 3). This report form will be forwarded to DWMS/BFBM staff who will determine the entities and partners to be contacted for follow-up. Partners could include: local health departments, local park authorities, academia, Rutgers Cooperative Extension(s), lake associations, watershed associations, DEP Watershed Ambassadors, and volunteers.

If follow-up is with a government entity concerning a public water body, DWMS/BFBM will coordinate any possible response monitoring and analysis as requested. If a report concerns a private waterbody, guidance will be provided to the owning entity regarding the proper response. If report is regarding a direct drinking water source the DEP DWSG will be contacted.

Upon initial reporting of a suspected HAB, one or more of the following field screenings will be performed by a qualified agency to verify whether a potential HAB is present. If field screenings verify a HAB may be present, a sample will be collected for further confirmatory analysis.

Figure 3. Quick Reporting Guide You can help!

If you observe what you think might be a HAB in a pond, lake, or stream, to report a suspected Harmful Algal Bloom, a call should be placed to the DEP Hotline at 1-877-WARNDEP (927-6337) or submitted through the WARN NJDEP mobile app (available via iTunes, Google Play or Windows Phone). In addition to contacting the DEP Hotline, please complete the HAB Reporting Form at: www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. You can also contact your local or county Health Department (see website below). Please note the exact location of the suspected HAB along with any details (e.g., date/time, bloom appearance and color, whether a swimming beach is nearby).

(<http://nj.gov/health/lh/directory/lhdselectcounty.shtml>), or county 24 hour hotlines (See Appendix A: County Notification List). Please report the exact location of the suspected HAB along with any details (e.g., date/time, bloom appearance and color, whether a swimming beach is nearby).



B. Visual Assessment

A visual assessment (Appendix E) will be performed by trained personnel upon initial reporting of a suspected HAB. This assessment is modified from a USEPA method as part of the National Lake Assessment (NLA). Procedures are outlined in: *USEPA. 2012 National Lakes Assessment: Field Operations Manual, Version 1.0, May 15, 2012. EPA 841-B-11-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC.* <https://www.epa.gov/national-aquatic-resource-surveys/nla>.

C. Screening

i. Cyanobacteria Presence

If visual assessment confirms a potential HAB, procedures to establish the presence of cyanobacteria taxa can be performed in the field or laboratory, including the following:

Field. The presence of phycocyanin pigment (unique to cyanobacteria taxa) is determined using a handheld field fluorometer. (See Appendices C-H for additional information and resources). If a field fluorometer is not available, a sample may be collected for laboratory screening. Sample collection for HABs follows the method for Phytoplankton Sampling, Chapter 6.10.1 of the NJDEP Field Sampling Procedures Manual, August 2005, <http://www.state.nj.us/dep/srp/guidance/fspm/>. Samples should be collected in amber glass bottles, refrigerated, and analyzed within 24 hours. Exact sample size, collection materials, holding times, and preservation should be confirmed with the laboratory, however, all collection procedures and preservation should comply with the minimum requirements of the analytical method.

Laboratory. A broad identification of taxa may be performed using microscopic equipment.

If cyanobacteria taxa are confirmed using either of these screening methods, toxins and/ or cell counts should be analyzed per below.

ii. Toxin Presence

An ABRAXIS microcystins test strip reading can be used to identify the presence of the total microcystin toxins (including –LR and other detectable congeners). Test strips for Cylindrospermopsin and Anatoxin –a. are also available; DEP BFBM is investigating the feasibility of their use for initial field identification of these two toxins. Qualitative microcystins test strip results will be validated, per the manufacturer’s instructions (Appendix G) in the following manner:

Qualitative Microcystins Test Strip Interpretation

- Control line not present/ Test line not present: invalid result
- Control line present/ Test line not present: concentration result is >10 µg/L (ppb)
- Control line present Moderate intensity/Test line present: concentration result is between 0 and 10 µg/L (ppb)
- If at any time, microcystin strip test results indicate the presence of microcystin, water samples will be collected for microcystin analysis in the laboratory.

It should be cautioned that the absence of microcystins does not indicate the absence of all toxins, such as cylindrospermopsin and anatoxin-a. If any other screening indicates the presence of a potential HAB, then lab analysis should be performed for the remaining toxins.

D. Confirmation Toxin Analysis

The following cyanotoxins will be analyzed to confirm presence after the initial screening:

Microcystins

Microcystins are a group of at least 80-100 toxin variants which share a cyclic heptapeptide structure and primarily affect the liver (hepatotoxin). Microcystins are the most widespread cyanobacterial toxins and can bioaccumulate in common aquatic vertebrates and invertebrates such as fish, mussels, and zooplankton. Microcystins are produced by *Microcystis*, *Anabaena*, *Planktothrix* (*Oscillatoria*), *Nostoc*, *Hapalosiphon*, *Anabaenopsis*, *Fischerella*, *Gloeotrichia*, *Nodularia*, and *Snowella lacustris*. Nodularin, which is structurally related to microcystin and has a similar mode of toxicity, has been isolated from only one species of cyanobacteria, *Nodularia spumigena*. Recent evaluation of carcinogenesis from microcystin exposure by the International Agency for Research in Cancer has determined that microcystin-LR is possibly carcinogenic to humans (Group 2B), and has been suggested to be a tumor promoter and linked to incidences of human liver and colon cancer.

Cylindrospermopsin

Cylindrospermopsin is usually produced by *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Anabaena bergii*, *Umezakia natans*, *Aphanizomenon flos-aquae* and *gracile*, *Anabaena lapponica* and *planctonica*, *Lyngbya wollei*, and *Raphidiopsis mediterranea* and *Raphidiopsis curvata*. The primary toxic effect of this toxin is irreversible damage to the liver. It also appears to have a progressive effect on several other vital organs. Effects of poisoning in humans included hepatoenteritis and renal insufficiency.

Anatoxin-a

Anatoxin-a binds to neuronal nicotinic acetylcholine receptors affecting the central nervous system (neurotoxins). There are multiple variants, including anatoxin-a, homoanatoxin-a, and anatoxin-a(s). Although other anatoxin(s) and homo-anatoxins exist, there is currently no toxicity data to definitively determine if they have the same health effects as anatoxin-a. These toxins are mainly associated with the cyanobacterial genera *Oscillatoria species*, *Cylindrospermum*, *Planktothrix spp.*, *Aphanizomenon spp.*, *Lyngbya* and species such as *Anabaena flos-aquae*, *A. planktonica*, *Cuspidothrix*, *Cylindrospermopsis*, *Dolichospermum*, *Microcystis*, *Phormidium*, *Anabaena flos-aquae*, *A. lemmermannii*, *Raphidiopsis mediterranea* (strain of *Cylindrospermopsis raciborskii*), *Tychonema* and *Woronichinia*. (USEPA's HABs website: <https://www.epa.gov/nutrient-policy-data/cyanohabs>)

i. Toxin Analysis Methods

Samples analyzed by DWMS/BFBM will use a microtiter plate Enzyme-Linked Immuno-Sorbent Assay (ELISA), EPA method 546, using an automated plate reader (Figure 4) and ABRAXIS kits (Sample Collection Reference Guide Methods in Appendix F and G respectively). This method was utilized by the USEPA as part of the National Lakes Assessment (NLA). QA/QC procedures are outlined in: *USEPA. 2009 (Final). Survey of the Nation's Lakes: Integrated Quality Assurance Project Plan. EPA/841-B-07-003. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.* <https://www.epa.gov/national-aquatic-resource-surveys/nla>.

- Microcystins (> 80 variants)
 - Method – ELISA (EPA [Method 546](#))
 - Detection level = 0.10 µg/l
- Cylindrospermopsin
 - Method - ELISA.
 - Detection level = 0.050 µg/l
- Anatoxin-a
 - Method – ELISA
 - Detection level = 0.10 µg/l

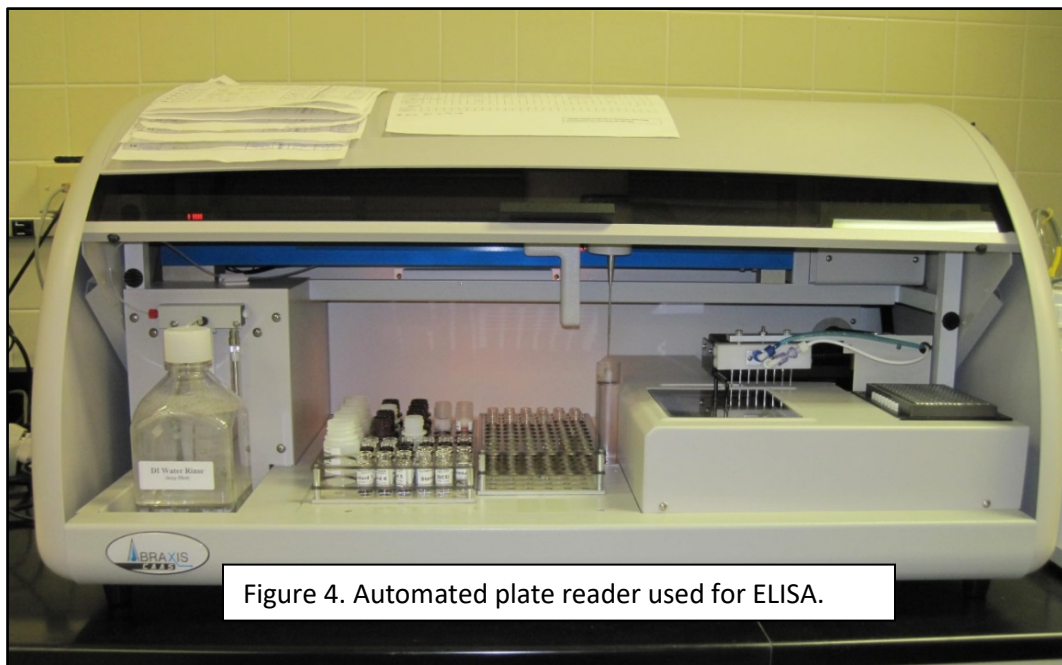


Figure 4. Automated plate reader used for ELISA.

For detection of cyanotoxins in drinking water, EPA developed [Method 544](#), a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for microcystins and nodularin (combined intracellular and extracellular), and [Method 545](#), a LC-ESI/MS/MS method for the determination of cylindrospermopsin and anatoxin-a. These methods, as well as Method 546 above are published in EPA's "Revisions to the Unregulated Contaminant Monitoring Rule (UCMR 4) for Public Water Systems and Announcement of Public Meeting" on December 20, 2016 (81 FR 92666). UCMR 4 includes Assessment Monitoring for a total of 30 chemical contaminants, including the cyanotoxins referred to here.

ii. Chlorophyll ‘a’ and cell counts

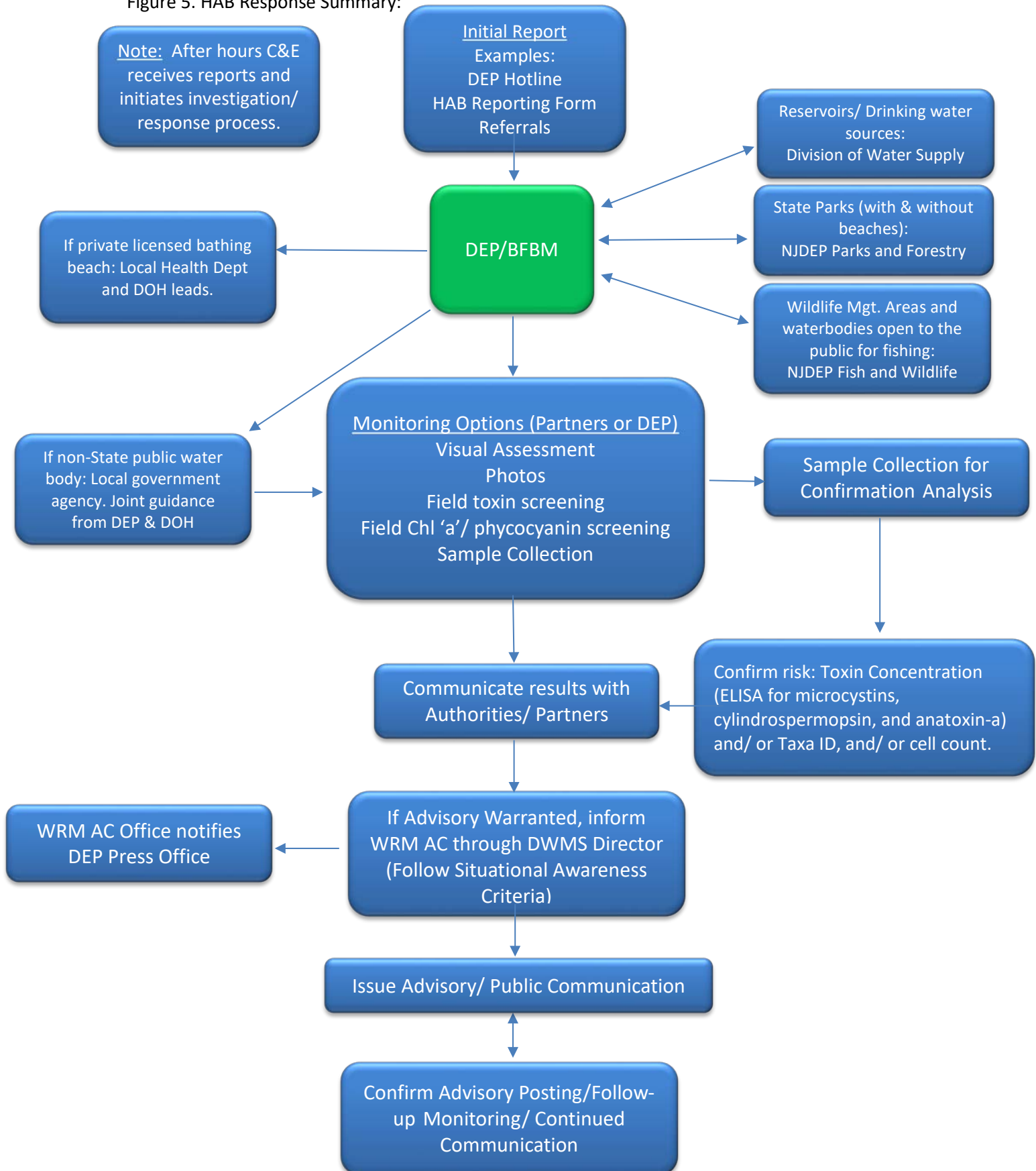
Algal concentrations in the water column are measured through Chlorophyll ‘a’ analysis. Chlorophyll “a” is contained in both green algae and cyanobacteria, both of which may be present in a bloom community at varying ratios. As a conservative estimate of possible health risk, it is assumed that higher concentrations of Chlorophyll ‘a’ increase the potential of higher cyanobacteria densities. Chlorophyll ‘a’ analysis (EPA Method 445.0) and/ or cell counts can be performed to inform advisories. WHO guidance for Chlorophyll ‘a’ and cell counts for moderate risk are Chlorophyll ‘a’ > 10 µg/l and cell count > 20,000 cells (Appendix H). WHO report is available at: http://www.who.int/water_sanitation_health/publications/srwe1/en/.

E. Response/ Actions

Depending on the ownership (public vs private) of the water body and its use, a variety of actions may be taken by DWMS/BFBM to communicate risk to the proper authority and the public. (Figure 5 summarizes the response flow)

- DEP DWSG will be alerted for HABs in a waterbody that is a direct source for drinking water.
- If reported at a State Park, the specific State Park Superintendent will be notified.
- If reported at a Wildlife Management Area, Fish and Wildlife will be contacted.
 - For drinking water sources and State owned recreational waterbodies, there will be joint communication and coordination regarding actions among DEP divisions.
- If the report concerns a potential HAB at another public water body, county/ local health agency and others (e.g. park commissions) as appropriate will be notified with joint guidance from DEP and DOH.
- If a report concerns a private waterbody, guidance will be provided to the owning entity regarding the proper response.
- If HAB poses a risk to livestock, appropriate NJ Department of Agriculture staff will be notified.
- BFBM will perform situational awareness in accordance with established internal DEP protocols.

Figure 5. HAB Response Summary:



F. Communication/ Continued Monitoring

If levels are above NJ Health Advisory Guidance for high risk and it is recommended that advisories be posted or recreational closures are warranted, situational awareness in accordance with established internal DEP protocols will be initiated. After initial HAB confirmation and actions, subsequent monitoring will likely be necessary and continue until the risk level subsides. Monitoring design including parameters, area of study, sample depth, frequency, and responsible entity will be determined on a case by-case basis. The monitoring design will consider the source of the HAB and potential for any exposure risks downstream of the originally reported water body including but not limited to: downstream drinking water sources, recreational and swimming areas, and livestock exposure. If monitoring is performed by DWMS/BFBM, results and/or additional information will continue to be communicated to authorities responsible.

After initial response and issuing of an advisory, it is the responsibility of the resource's authority (e.g. Fish and Wildlife, local health department) to communicate status to DWMS throughout the HAB event, until the advisory is ultimately lifted.

5. CYANOBACTERIAL HARMFUL ALGAL BLOOM ADVISORIES

Advisories are based on the recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure. Advisories are intended to be protective for a range of exposures and are probably highly conservative (i.e., protective) for the exposures most likely to occur. When posting advisories, it is recommended to err on the side of caution to avoid unnecessary risk to the public. These advisories may be altered to reflect the exact nature and extent of a specific HAB occurrence.

Danger/ High risk advisories will consider the recreational use of the water body, the extent of the bloom, as well as the magnitude of the exceedance of the cell counts and toxin levels above the NJ Health Advisory Guidance Levels.

Recommended wording for advisories is as follows:

Warning - Avoid Contact and Ingestion (Humans and Animals)

A suspected Harmful Algal Bloom is present which can produce toxins harmful to humans and animals. People, pets, and livestock should avoid contact and ingestion. Avoid swimming, wading, and watersports. Fish caught in this waterbody should not be eaten.



Danger/ High Risk - No Contact and Ingestion (Humans and Animals)

A confirmed Harmful Algal Bloom is present with levels quantified at or above the NJ Health Advisory Guidance. There should be no contact with the water including, but not limited to, swimming, wading, and watersports. Fish caught in this waterbody should not be eaten. Pets and livestock should not contact or ingest the water.



NOTE: A printable version of both of these HAB signs can be found on the web page below:

<http://www.state.nj.us/dep/wms/bfbm/advlanguage.html>

Guidance for lifting advisories and/ or re-opening bathing beaches.

If the above advisories are posted or result in a waterbody closure, the following guidance for lifting advisories and/ or re-opening is recommended:

Warning – Avoid Contact

- Continue lab analysis to confirm levels remain below thresholds. Analysis frequency to be determined on a case by case basis.
- Postings should remain in effect until HAB has visually dissipated and laboratory analysis confirm levels remain below thresholds.
- When advisory is lifted, continue daily observation of the lake and document findings. If a HAB re-occurs, then follow up laboratory analysis is required.

Danger – No Contact

- If HAB is present with levels quantified at or above the NJ Health Advisory Guidance, the no contact/ closure should not be lifted until two (2) subsequent analyses are below thresholds.
- When advisory is lifted, continue daily observation of the lake and document findings. If a HAB re-occurs, then follow up laboratory analysis is required.
- Test strip testing for the toxic group of microcystins should be done on a weekly basis; if a HAB is observed any day, a test strip will be performed immediately.
- Licensed bathing areas, same as above, however, if an HAB is observed then automatic closure of the swimming area until thorough testing is conducted and no levels are detected above thresholds.
- Any re-opening of bathing areas will be confirmed with DOH before implementation.

6. RESEARCH

DEP's DSREH, in coordination with the DWMS/BFBM, will provide technical consultation regarding HAB bloom response as well as conduct literature and applied research concerning:

- Human health exposure and impacts, including risks of consumption of fish including commonly caught game fish.
- Establishing thresholds for recreational risk and source drinking water risk
- Defining acceptable parameters and methods for assessing risk
- Researching new developments in HAB monitoring and analysis

Literature research will include HAB monitoring and response strategies established in other states as well as current USEPA guidance, USGS, academic, and other research.

A cyanobacterial HAB research and information needs plan will be developed, which may include applied research related to:

- Technology
 - Investigate the feasibility and reliability of new analyses, monitoring equipment and surveillance equipment, such as:
 - Use of satellite imagery, monitoring aerial unmanned vehicles, and other aircraft-based sensor technology.
 - Flowcytometer and Luminex Assays as potential methods.
 - Molecular PCR and qPCR techniques for identification and quantification.
- Pilot Studies
 - Coordinate with academia and other local agencies to develop enhanced monitoring and detection techniques.
- Predictive Tools/Prevention
 - Use water quality data, bathymetry, weather/ climate, land use, and other information to predict possible events or prevent events through lake management.
- Treatment
 - Effective treatment for prevention and elimination of HABs (communities and toxins).

New information and enhancements will be added to the DWMS HABs website and/ or this Strategy as it becomes available.

7. OUTREACH and COMMUNICATION

DEP will continue its efforts to provide up- to- date and easily accessible information, both within the Department, other State and local agencies, as well as to the public. Communication mechanisms presently being pursued for feasibility and implementation include, but are not limited to:

- Enhance DEP website information on HABs.
- Continue communication/ coordination on HABs with the New Jersey Water Monitoring Council (NJWMC) which serves as a statewide body to promote and facilitate the coordination, collaboration and communication of scientifically sound, ambient water quality and quantity information to support effective water resource management.
- Communication/coordination with County Environmental Health Act (CEHA) program representatives from DEP and each county.
- Training and information exchange for Departmental programs, partners and the public, such as in-person training, webinars and web- based training.
- Development and/or use of existing Smart phone apps, such as Bloomwatch, for identifying, reporting, and communicating potential HAB concerns.
- Enhance information that would be accessible at NJ State Parks through physical signage, informational material, increased information on individual park websites, etc.
- Partnering with other state agencies in the region to adapt existing communication efforts for New Jersey.
- Investigate use of the new Center for Disease Control's One Health Harmful Algal Bloom System (OHHABS). The One Health Harmful Algal Bloom System (OHHABS) is a voluntary reporting system available to state and territorial public health departments and their designated environmental health or animal health partners. It collects data on individual human and animal cases of illnesses from HAB-associated exposures, as well as environmental data about HABs. The goal of OHHABS is to collect information to support the understanding and prevention of HABs and HAB-associated illnesses. DOH is the lead in exploring State participation in this effort.

8. References

1. NJDEP. Division of Science, Research and Environmental Health. Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin –A. 2017.
2. USEPA. Developing a Cyanobacteria Monitoring & Bloom Watch Program | EPA & Minnesota Sea Grant. November 9, 2016.
3. USEPA's HABs website: <https://www.epa.gov/nutrient-policy-data/cyanoHABs>.
4. USEPA. Draft Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. EPA 822-P-16-002. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC. 2016.
5. USEPA. (Final). Survey of the Nation's Lakes: Integrated Quality Assurance Project Plan. EPA/841-B-07-003. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC. 2009.
6. USEPA. Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water. Office of Water (4606M). EPA 815-R-15-010. June 2015.
7. USGS. Graham, J.L., Dubrovsky, N.M., and Eberts, S.M., 2016, Cyanobacterial harmful algal blooms and U.S. Geological Survey science capabilities: U.S. Geological Survey Open-File Report 2016–1174, 12 p., <https://pubs.er.usgs.gov/publication/ofr20161174>.
8. World Health Organization. Guidelines for Safe Recreational Water Environments. 2009. http://www.who.int/water_sanitation_health/publications/srwe1/en/.

Links to information websites including CDC, EPA, WHO can be found at the DWMS HAB webpage: www.state.nj.us/dep/wms/HABS.html .

Appendix A

Workgroup Members/Communications

New Jersey Harmful Algal Bloom (HAB) Workgroup (as of 11/8/2017)

DEP DWMS

Leslie McGeorge
Victor Poretti
Tom Miller
Dean Bryson
Alena Baldwin-Brown

DEP WRM

Matt Maffei

DEP DSREH
Robert Newby
Gloria Post
Nick Procopio
Tom Atherholt (retired)

DEP Water Supply and Geoscience

Linda Bonnette
Katrina Angarone
Kevin Giberson

DEP State Park Service

Dan Bello (retired)
Vincent Bonica, Southern Region
Dave Donnelly, Central Region
Blanca Chevestt, Northern Region

DEP Fish and Wildlife

Lisa Barno, Freshwater Fisheries
Jan Lovy, Office of Fish and Wildlife Health
and Forensics

DEP Office of Quality Assurance

Marc Ferko

DOH Division of Epidemiology, Environmental and Occupational Health/Consumer, Environmental and Occupational Health Service (CEOHS)

Loel Muetter
Jessie Gleason
Tim Smith (retired)

DOH Division of Epidemiology, Environmental and Occupational Health/ Communicable Disease Service (CDS)

Deepam Thomas
Rebecca Greeley
Barbara Carothers

Department of Agriculture/ Division of Animal Health

Nicole Lewis

Appendix A Continued

Communication (*Workgroup Members)

DEP

DEP Hotline - 877-WARN-DEP (877-927-6337) <http://www.nj.gov/dep/warndep.htm>

Division of Water Monitoring and Standards*

<http://www.nj.gov/dep/wms/>
www.state.nj.us/dep/wms/HABS.html

Bureau of Freshwater and Biological Monitoring (BFBM)*

609-292-0427

njcyanoabs@dep.nj.gov
www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

DEP Division of Science, Research and Environmental Health*

609-984-6070

<http://www.nj.gov/dep/dsr/>

DEP Division of Water Supply and Geoscience*

609-292-7219

watersupply@dep.nj.gov
<http://www.nj.gov/dep/watersupply/>

DEP Division of Fish & Wildlife *

609-292-2965

<http://www.nj.gov/dep/fgw/>

DEP State Park Service*

<http://www.nj.gov/dep/parksandforests/>

Southern Region 609-704-1951

Jurisdiction: Wharton State Forest, Atsion State Park, Bass River State Forest, Belleplain State Forest, Parvin State Park

Central Region 908-236-2043

Jurisdiction: Cheesequake State Park, Round Valley Recreation Area, Spruce Run Recreation Area

Northern Region 973-786-5210

Jurisdiction: High Point State Park, Hopatcong State Park, Ringwood State Park, Stokes State Forest, Swartswood State Park, Wawayanda State Park

DEP Compliance and Enforcement/ Division of Water and Land Use Enforcement

<http://www.nj.gov/dep/enforcement/dwlue.html>

609-984-2011

Bureau of Water Compliance & Enforcement-Northern

973-656-4099

Jurisdiction: Counties of Bergen, Essex, Hudson, Hunterdon, Morris, Passaic, Somerset, Sussex, and Warren

Bureau of Water Compliance & Enforcement-Central

609-292-3010

Jurisdiction: Counties of Mercer, Middlesex, Monmouth, Ocean, and Union

Bureau of Water Compliance & Enforcement-Southern

856-614-3655

Jurisdiction: Counties of Atlantic, Burlington, Camden, Cape May, Cumberland, Gloucester, and Salem

DEP Office of Quality Assurance*

(609) 292-3950

<http://www.nj.gov/dep/enforcement/oga.html>

New Jersey Department of Health (DOH)

AFTER HOURS EMERGENCY CONTACT

609-392-2020

Public Health and Food Protection Program*

<http://www.nj.gov/health/phss/index.shtml>

609-826-4935

Consumer, Environmental and Occupational Health Service*

<http://www.nj.gov/health/ceohs/index.shtml>

Public Recreational Bathing Project*

<http://www.nj.gov/health/ceohs/sanitation-safety/environmental/>

Local Health Department Directory

<http://nj.gov/health/lh/directory/lhdselectcounty.shtml>

New Jersey Department of Agriculture

Division of Animal Health/ New Jersey Animal Emergency Response*

609-671-6400

<http://www.nj.gov/agriculture/divisions/ah/>

Appendix B

Local and county Health Department notification list:

<http://nj.gov/health/lh/directory/lhdselectcounty.shtml>

In New Jersey, every municipality is required to be served by a local health department that meets the requirements of state public health laws and regulations. The local health departments listed in this directory are recognized by the New Jersey Department of Health as the provider of public health services for those municipalities within their jurisdiction.

Should you have questions about available public health services or concerns about health conditions within a particular municipality, please use this directory to obtain important information about how to contact the local health department. In cases where a municipality is temporarily without the services of a local health department, you will be provided with contact information for that municipality's administrative offices.

To begin your search, select a county or municipality from the link above. You may also print [Directory of Local Health Departments in New Jersey](#) [PDF 163k] OR [Directory of After Hour Emergency Contact Phone Numbers for Local Health Departments](#) [PDF 76k].

APPENDIX C
DEP/DWMS/BFBM Fact Sheets

- 1. Cyanobacterial Harmful Algal Blooms (HABs)**
- 2. Cyanobacteria Harmful Algal Blooms (HABs) and
Cyanotoxins: Recreational Exposure and Health Effects**



Cyanobacterial Harmful Algal Blooms (HABs)

What are Cyanobacteria?

Cyanobacteria are a type of bacteria capable of photosynthesis. Although they are not true algae, they are often referred to as “blue-green algae”. Cyanobacteria frequently impart off-tastes and odors to the water in which they grow, and sometimes they produce toxins that can be harmful to the health of humans and other animals. Although problems related to cyanobacteria most often occur in freshwaters (lakes and streams), cyanobacteria can also be found in marine waters.



What are Cyanobacterial Harmful Algal Blooms (HABs)?

A cyanobacterial Harmful Algal Bloom (HAB) is the name given to the excessive growth, or “bloom”, of cyanobacteria, some of which can produce one or more types of potentially harmful toxins. HABs can occur under suitable environmental conditions of light, temperature, nutrients, and calm water. These “blooms” often result in a thick coating or “mat” on the surface of a waterbody, often in late-summer or early fall.




How do I identify a Cyanobacterial Harmful Algal Bloom (HAB)?

A cyanobacterial HAB often looks like a layer of bright bluish-green or white paint on the water surface. Other evidence of a potential cyanobacterial HAB could be discolored or pea-green colored water, parallel streaks, or green dots/globs in the water. It is important to note that some blooms are due to common green algae and not cyanobacteria and, when present, cyanobacteria do not always produce cyanotoxins. Below are some photographs of cyanobacterial HABs and also photographs of algal mats, surface films, plant pollen, or harmless plants that may resemble, but are not cyanobacterial HABs.

Cyanobacterial Harmful Algal Bloom (HAB) photos

	
HAB	sampling a bloom for analysis

Non-HAB (harmless look-alikes) photos

		
Algal mat (algae or water plants that have collected on the surface)	Muck (a mixture of organic material on the surface or banks)	Duckweed (a tiny floating plant with true leaves)

Are Cyanobacterial Harmful Algal Blooms (HABs) harmful?

Avoid contact if you see what you think might be such a HAB, and report its location to one of the contacts listed below. Because animals can be harmed by drinking from waterbodies during a cyanobacterial HAB event, keep pets and domestic animals away from waters that you suspect may have a HAB. If a HAB has been identified in a body of water used for drinking or recreation, signs may be posted by local or county authorities.

Should I swim?

You should not swim or conduct any activity which might result in direct contact with the water when a suspected HAB is present.

Should I eat the fish?

You should not eat fish or shellfish from a waterbody experiencing a HAB.

Should I drink the water?

Never consume untreated surface water (e.g., lakes, ponds, or streams), especially if a HAB is suspected.

What is the NJ Department of Environmental Protection doing?

NJDEP's Ambient Lakes Monitoring Program routinely monitors a network of freshwater lakes in NJ for the presence of HABs, as well as the chemical and physical parameters that are conducive to HAB growth <http://www.state.nj.us/dep/wms/bfbm/lakes.html>. Sampling and analyses for the presence of the most common cyanotoxins in these lakes was initiated in 2013, and a response plan is in place for reported blooms. NJDEP is also partnering with Rutgers University to do a pilot study investigating contributors to cyanotoxins in selected lakes.

If an algal bloom occurs in freshwater that is used for drinking water, the NJDEP partners with the drinking water supplier to test if cyanobacteria and cyanotoxins are present, so that appropriate adjustments to drinking water treatment are implemented, if necessary.

You can help!

If you observe what you think might be a HAB in a pond, lake, or stream, to report a suspected Harmful Algal Bloom, a call should be placed to the DEP Hotline at 1-877-WARNDEP (927-6337) or submitted through the WARN NJDEP mobile app (available via iTunes, Google Play or Windows Phone). In addition to contacting the DEP Hotline, please complete the HAB Reporting Form at: www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. You can also contact your local or county Health Department (see website below). Please note the exact location of the suspected HAB along with any details (e.g., date/time, bloom appearance and color, whether a swimming beach is nearby).

Contacts

NJDEP Hotline: 1-877-WARNDEP (1-877-927-6337) <http://www.nj.gov/dep/warndep.htm>

NJDEP Bureau of Freshwater & Biological Monitoring (609-292-0427)

njcyanohabs@dep.nj.gov

www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

<http://www.state.nj.us/dep/wms/bfbm>

NJDOH Public Health Sanitation and Safety Program (609-826-4941)

<http://nj.gov/health/phss>

Local and county Health Departments in New Jersey

<http://www.state.nj.us/health/lh/directory/lhdselectcounty.shtml>

For questions regarding drinking water, please contact your local water supplier or

NJDEP Division of Water Supply and Geoscience (609-292-7219)

<http://www.nj.gov/dep/watersupply>

Additional Information

NJDEP Division of Water Monitoring and Standards

Harmful Algal Bloom Website: www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

U.S. Environmental Protection Agency (EPA) Cyanobacterial Harmful

Algal Blooms: (including links to other states' information)

<https://www.epa.gov/nutrient-policy-data/cyanohabs>

Rutgers New Jersey Agricultural Experiment Station

Blue-green Algae in Waterways:

<http://njaes.rutgers.edu/pubs/fs1216/>

NY Department of Environmental Conservation

Blue-green Harmful Algal Blooms:

<http://www.dec.ny.gov/chemical/77118.html>

National Oceanographic and Atmospheric Administration (NOAA)

<http://oceanservice.noaa.gov/news/features/feb12/cyanobacteria.html>

Cyanobacteria Harmful Algal Blooms (HABs) and Cyanotoxins: Recreational Exposure, Health Effects and Guidance Levels

What are Cyanobacteria and Cyanotoxins?

Cyanobacteria are a type of bacteria capable of photosynthesis. Although they are not true algae, they are often referred to as “blue-green algae”. A cyanobacterial Harmful Algal Bloom (CyanoHAB) is an excessive growth, or “bloom”, of cyanobacteria, some of which can produce one or more types of potentially harmful toxins (cyanotoxins). HABs can occur under suitable environmental conditions of light, temperature, nutrients, and calm water. These “blooms” often result in a thick coating or “mat” on the surface of a waterbody, often in late-summer or early fall. People, pets, livestock and/or wildlife can be exposed to CyanoHABs by coming in contact with or ingesting water that is experiencing a CyanoHAB bloom. If a waterbody is suspected of having a CyanoHAB, people, pets and livestock should avoid contact with and not consume the water, and fish caught in the affected waterbody should not be consumed. A separate fact sheet providing general information about CyanoHABs is available at: www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. The fact sheet here provides detailed information on CyanoHAB Recreational Exposures, Health Effects, and the NJ recreational advisory guidance levels.

What are the potential human health impacts from recreational exposure to cyanobacteria and related toxins?

During recreational activities (e.g. swimming, wading, and watersport activities including jet skiing, kayaking, wind surfing, and paddleboarding), exposure to cyanobacteria and the toxic chemicals (cyanotoxins) produced by these organisms can occur. Recreational exposure can occur from accidental or deliberate ingestion of water, direct skin contact, or inhalation of water droplets.

Adverse health effects of cyanotoxins include allergic-like reactions (e.g., rhinitis, asthma, eczema, and conjunctivitis), flu-like symptoms, gastroenteritis, respiratory irritation, skin rashes, and eye irritation. More serious adverse health effects may include liver toxicity and neurological effects. Studies in laboratory animals suggest the possible involvement of some cyanotoxins in tumor formation.

The table below summarizes the health effects caused by the most common cyanotoxins and the species of cyanobacteria that are capable of producing them.



The Primary Cyanotoxins and their Health Effects (Based on USEPA’s table located at their website on HABS: <https://www.epa.gov/nutrient-policy-data/cyanohabs>)

Cyanotoxin	Health Effects in Humans	Cyanobacteria that can produce the toxin
Microcystin-LR	Abdominal pain, headache, sore throat, nausea and vomiting, dry cough, diarrhea, blistering around the mouth, pneumonia, liver toxicity.	<i>Microcystis</i> , <i>Anabaena</i> , <i>Nodularia</i> , <i>Planktothrix</i> , <i>Fischerella</i> , <i>Nostoc</i> , <i>Oscillatoria</i> , and <i>Gloeotrichia</i>
Cylindrospermopsin	Fever, headache, vomiting, bloody diarrhea, liver and kidney toxicity	<i>Cylindrospermopsis raciborskii</i> , <i>Aphanizomenon flos-aquae</i> , <i>Aphanizomenon gracile</i> , <i>Aphanizomenon ovalisporum</i> , <i>Umezakia natans</i> , <i>Anabaena bergii</i> , <i>Anabaena lapponica</i> , <i>Anabaena planctonica</i> , <i>Lyngbya wollei</i> , <i>Raphidiopsis curvata</i> , and <i>Raphidiopsis mediterranea</i>
Anatoxin-a group	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death, neurotoxin.	<i>Chrysothrix</i> (<i>Aphanizomenon</i>) <i>ovalisporum</i> , <i>Cuspidothrix</i> , <i>Cylindrospermopsis</i> , <i>Cylindrospermum</i> , <i>Dolichospermum</i> , <i>Microcystis</i> , <i>Oscillatoria</i> , <i>Planktothrix</i> , <i>Phormidium</i> , <i>Anabaena flos-aquae</i> , <i>A. lemmermannii</i> <i>Raphidiopsis mediterranea</i> (strain of <i>Cylindrospermopsis raciborskii</i>), <i>Tychonema</i> and <i>Woronichinia</i>

What recreational HAB guidance levels are available?

EPA is currently developing recreational threshold values for one or more of these cyanotoxins, while a number of states, as well as the World Health Organization (WHO), have derived their own “action levels” or health advisory guidelines based on cyanobacteria cell counts and/or concentrations of the more toxic, commonly-occurring cyanotoxins.

The Bureau of Freshwater and Biological Monitoring of the NJ Department of Environmental Protection (NJDEP) has developed the laboratory capability to measure levels of three of the most toxic, commonly observed cyanobacterial toxins in NJ’s freshwaters, namely microcystins (suite of microcystin congeners), cylindrospermopsin, and anatoxin-a.

NJDEP’s Division of Science, Research and Environmental Health (DSREH) has reviewed the cyanotoxin guidance values developed by WHO and various states, as well as relevant scientific publications, and has developed guidance values for these three toxins (see below) which are applicable to recreational exposure. These values will be used by NJ to provide advice on recreational activities in response to HABS.

What are the recommended recreational action levels and health advisory guidance levels for New Jersey waters?

Action level based on cyanobacterial cell counts

Low concentrations of cyanobacteria may cause allergenic and/or irritant effects to a portion of an exposed population. These effects are caused by endotoxins (mainly the lipopolysaccharide component of the cyanobacterial cell wall) rather than cyanotoxins. Therefore, county or local authorities may wish to post precautionary advisories for freshwater lakes or ponds in which cyanobacterial blooms are suspected through visual or other screenings, until confirmation analysis is performed.



It is recommended that if the cyanobacterial cell count equals or exceeds 20,000 cells/ml in an area where primary recreational contact is likely to occur, county or local authorities should post advisory signs. When cell counts exceed this level, or other visual or screening assessments indicate a suspected bloom, monitoring for cyanotoxins should be initiated. This recommendation is based on the WHO (2003a) guidance (i.e. $\geq 20,000 - 100,000$ cells/ml is categorized as moderate risk) described in detail in the full DSREH document: Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin-A.

Health advisory guidance levels for individual cyanotoxins

The following guidance values are recommended for recreational exposure to individual cyanotoxins or, in the case of microcystins, toxin groups. The bases for these values is provided in the NJ Action Level document within the overall Response Strategy which can be located at:

www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

- Microcystins (as total including –LR and other detectable congeners): 3 $\mu\text{g/L}$
- Cylindrospermopsin: 8 $\mu\text{g/L}$
- Anatoxin-a: 27 $\mu\text{g/L}$

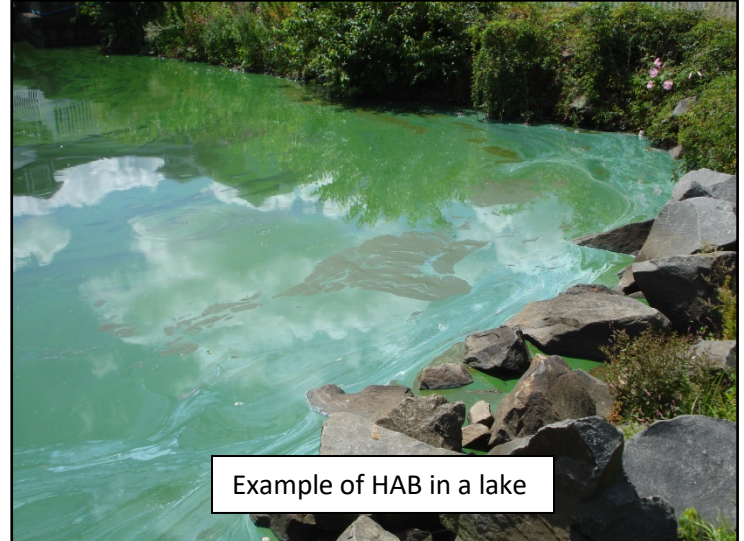
These concentrations are intended to be protective during short-term exposures, such as multiple days of swimming during the swimming season, for the more sensitive sub-population of children. The values are probably highly conservative (i.e., protective) for the degree of exposure most likely to occur. The uncertainties in the risk estimates underlying the development of these values, as well as the inherent uncertainty in the time course and location of the toxins in any given waterbody, should be considered when providing advice to the public regarding recreation in affected waterbodies.

What other conditions besides threshold level exceedances could result in advisories or warnings?

Upon initial reporting of a suspected HAB, one or more of the following field screenings will be performed by a qualified organization to verify whether or not a potential HAB is present. If field screenings verify that a HAB may be present, a sample will be collected for further confirmatory analysis.

Visual Assessment

This approach is used to provide visual assessment of lake conditions and land use as well as HAB presence. This assessment is available in the Cyanobacterial Harmful Algal Blooms (HABs) Freshwater Recreational Response Strategy



(www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html) and is modified from a USEPA method as part of the National Lake Assessment (NLA). Procedures are outlined in: *USEPA. 2012 National Lakes Assessment: Field Operations Manual, Version 1.0, May 15, 2012. EPA 841-B-11-003. U.S. Environmental Protection Agency, Office of Water, Washington, DC.*

Cyanobacteria Presence

If visual assessment confirms a potential HAB, the presence of cyanobacteria species can be confirmed in two ways, if equipment is available: 1. a broad identification of individual cyanobacteria may be performed using microscope equipment; or 2. the presence of phycocyanin pigment (unique to cyanobacteria) may be determined using a handheld field fluorometer.

Toxin Presence Screening

A microcystins field-kit test strip reading can be used to identify the presence of microcystins (other toxin test strips are now available; NJDEP is investigating the feasibility of use).

What are appropriate responses if the NJ recreational water advisory guidance levels are exceeded?

NJ thresholds are developed for human exposures only. They do not apply to pets, livestock, or other animals, and they do not apply to fish consumption. Contact should be avoided by livestock and pets when evidence of HABs or their toxins are present.

Example Advisories:

Warning - Avoid Contact and Ingestion (Humans and Animals)

A suspected Cyanobacteria bloom is present which can produce toxins harmful to humans and animals. People, pets, and livestock should avoid contact and ingestion. Exposure can include swimming, wading, and watersports. Fish caught in this waterbody should not be eaten

Danger/ High Risk - No Contact and Ingestion (Humans and Animals)

A confirmed Harmful Algal Bloom is present with levels quantified at or above the NJ Health Advisory Guidance. There should be no contact with the water including, but not limited to, swimming, wading, and watersports. Fish caught in this waterbody should not be eaten. Pets and livestock should not contact or ingest the water.

What effects can HABs and cyanotoxins have on pets, livestock and aquatic organisms, such as fish?

Livestock, pets, and other wildlife can become ill or die after drinking water contaminated by HAB toxins. Algal blooms may cause off-flavor and objectionable odors in fish, and lead to fish kills and decrease fish production because of oxygen depletion.

How are people or animals treated that have been exposed to cyanobacterial blooms?

The Center for Disease Control (CDC) states that if you or your pet comes in contact with a cyanobacteria, wash yourself and your pet thoroughly with fresh water. If you or your pet swallow water from where there is a harmful algae bloom, call your doctor, a Poison Center, or a veterinarian. Call a veterinarian if your animal shows any of the following symptoms of cyanobacteria poisoning: loss of appetite, loss of energy, vomiting, stumbling and falling, foaming at the mouth, diarrhea, convulsions, excessive drooling, tremors and seizures, or any other unexplained sickness after being in contact with water. CDC website: <http://www.cdc.gov/habs/materials/factsheets.html>.

You can help!

If you observe what you think might be a HAB in a pond, lake, or stream, to report a suspected Harmful Algal Bloom, a call should be placed to the DEP Hotline at 1-877-WARNDEP (927-6337) or submitted through the WARN NJDEP mobile app (available via iTunes, Google Play or Windows Phone). In addition to contacting the DEP Hotline, please complete the HAB Reporting Form at: www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. You can also contact your local or county Health Department (see website below). Please note the exact location of the suspected HAB along with any details (e.g., date/time, bloom appearance and color, whether a swimming beach is nearby).

Contacts

NJDEP Hotline: 1-877-WARNDEP (1-877-927-6337)

<http://www.nj.gov/dep/warndep.htm>

NJDEP Bureau of Freshwater & Biological Monitoring (609-292-0427)

njcyanoabs@dep.nj.gov

www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

<http://www.state.nj.us/dep/wms/bfbm>

NJDOH Public Health Sanitation and Safety Program (609-826-4941)

<http://www.nj.gov/health/ceohs/sanitation-safety/environmental/>

Local and county Health Departments in New Jersey

<http://www.state.nj.us/health/lh/directory/lhdselectcounty.shtml>

For questions regarding drinking water, please contact your local water supplier or NJDEP Division of Water Supply and Geoscience (609-292-7219)

<http://www.nj.gov/dep/watersupply>

Additional Resources:

NJDEP Division of Water Monitoring and Standards Harmful Algal Bloom Website:

www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

USEPA's website on HABs, including Draft Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin: <https://www.epa.gov/nutrient-policy-data/cyanoabs>.

Harmful Algal Blooms and Hypoxia Comprehensive Research Plan and Action Strategy: An Interagency Report, National Science and Technology Council, Feb. 2016. <https://www.epa.gov/nutrient-policy-data/guidelines-and-recommendations>

USGS. Graham, J.L., Dubrovsky, N.M., and Eberts, S.M., 2016, Cyanobacterial harmful algal blooms and U.S. Geological Survey science capabilities: U.S. Geological Survey Open-File Report 2016-1174, 12 p., <https://pubs.er.usgs.gov/publication/ofr20161174>.

APPENDIX D
DEP/DWMS/BFBM HAB Reporting Form

Suspected Harmful Algal Bloom Report - Freshwater



(Printable PDF version - Electronic version also available at <http://www.nj.gov/dep/wms/bfbm/CyanoHABHome.html>.)

Cyanobacteria, which are sometimes referred to as blue-green algae, are tiny organisms that are naturally present in lakes and streams. Under certain conditions, they can spread quickly and form dense blooms in the waterbody. These blooms can be problematic since some cyanobacteria species produce toxins that are harmful to humans, pets, and wildlife. A general fact sheet on Cyanobacterial harmful algal blooms is available at: <http://www.nj.gov/dep/wms/bfbm/download/HABsFactSheet.pdf>.

Please complete the following information to report a potential Harmful Algal Bloom (HAB) in a freshwater waterbody (e.g., lake, river, stream).

First Name:*	Last Name:*	Enter at least one of the following 2 fields: *	Phone Number:	Email:
<input type="text"/>	<input type="text"/>		<input type="text"/>	<input type="text"/>
Affiliation:	Water Body Name:	Date Bloom Observed (MM/DD/YYYY):*		
<input type="text"/>	<input type="text"/>	<input type="text"/>		

*****Please fill out the remainder of form to the best of your knowledge*****

Waterbody Location & Contact Information:

Street Address:	Closest Cross Street:	City:	County:
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
State:	Postal/Zip Code:	Latitude:	Longitude:
New Jersey	<input type="text"/>	<input type="text"/>	<input type="text"/>

To find your latitude and longitude, you can access a maps website/application on your computer or smart phone, i.e. [Google Maps](#) or [Apple Maps](#).

Was the bloom reported to DEP Hotline (1-877-927-6337) or via the WARN NJDEP mobile app?



If yes, please provide the Case # (12 digit #):

- ☐ Yes
☐ No
☐ Unknown

Was the bloom reported to any other government agency?

If yes, what agency (DEP, DOH, local health dept, local gov't, etc.)?

- ☐ Yes
☐ No
☐ Unknown

Does waterbody have a bathing beach?

Public or privately owned?

Owner Name (state parks, lake association, beach club, municipality, county, etc.):

- ☐ Yes
☐ No
☐ Unknown

- ☐ Public
☐ Private
☐ Unknown

Is the waterbody a source for public drinking water?

- ☐ Yes
☐ No
☐ Unknown

Specify water system name if known:

If "yes", is there any additional information on water supply available (e.g. distance of bloom from drinking water intake)?

- ☐ Yes
☐ No
☐ Unknown

Please describe:

Bloom Description and Sampling Information:

Where on the lake/waterbody do you see the bloom?

- ☐ Shoreline
☐ Other parts of waterbody
☐ Both

Which sections of the lake/ waterbody have a bloom? (Select all that apply)

- ☐ North
☐ South
☐ East
☐ West

How large is the extent of the bloom?

- ☐ Larger than a football field
☐ Between a football field & a tennis court
☐ Between a tennis court & a car
☐ Smaller than a car
☐ Unknown

Other bloom appearance (check all that apply):
(See bottom of form for example images.)

- ☐ Spilled paint
☐ Pea soup
☐ Green streaks parallel to shore line
☐ Large concentration of "green dots"

Was there a foul smell associated with the bloom (e.g., septic, rotten, fishy, earthy)?

- ☐ Yes
☐ No
☐ Unknown

Please describe:

Can you see a surface scum (an accumulation of algae at the surface) or algae floating near the water surface (algae at the surface can look like green/blue thick foam or spilled paint).
(See bottom of form for example images.)

- ☐ Yes
☐ No
☐ Unknown

Did you notice any colors in the water column?

- ☐ Yes
☐ No

Please describe the color(s) you see:

Is the bloom near a bathing beach, boat ramp, marina, or other recreational access?

- ☐ Yes
☐ No
☐ Unknown

Please describe the location:

Describe any other recreation:

Is there a fish kill or impacts to other wildlife?

- ☐ Yes
☐ No
☐ Unknown

Please describe:

Were samples taken?

- ☐ Yes
☐ No
☐ Unknown

If yes, what types of samples, when and where were they collected, and where were they sent for analysis, results, etc?

Do you know if other water quality information is available (e.g. pH, dissolved oxygen)?

- ☐ Yes
☐ No
☐ Unknown

Please specify:

Please scan & email completed form to njcyanoabs@dep.nj.gov or fax it to (609) 633-1095. Please email any available photos of the HAB to njcyanoabs@dep.nj.gov.

For questions about this form, please contact us at njcyanoabs@dep.nj.gov or (609) 292-0427.

***Required Field**

DEP ID# (for Internal Use Only):



Pea Soup

Photo Credit: NJDEP



Spilled Paint

Photo Credit: NJDEP



Green Streaks

Photo Credit: NJDEP



Green Dots

Photo Credit: NJDEP



Surface Scum

Photo Credit: NJDEP



Green/Blue Foam

Photo Credit: NJDEP

APPENDIX E
DEP/DWMS/BFBM HAB Visual Assessment Form

LAKE ASSESSMENT FORM

SITE ID: _____ DATE: ____/____/____

Lake Name: _____ Form Completed By: _____

LAKE/CATCHMENT SITE ACTIVITIES AND DISTURBANCES OBSERVED

(Intensity: **Blank=Not Observed**, L=Low, M=Moderate, H=Heavy, P=Present)

Residential	Recreational	Agricultural	Industrial	Lake Management
L M H Residences	L M H Hiking/Walking Trails	L M H Cropland	L M H Industrial Plants	P Fish Stocking
L M H Maintained Lawns	L M H Parks, Campgrounds	L M H Pasture	L M H Mines/Quarries	P Chemical Treatment*
L M H Construction	L M H Primitive Parks, Camping	L M H Livestock Use	L M H Odors	P Liming
L M H Pipes, Drains	L M H Golfing/Resorts	L M H Orchards	L M H Power Plants	P Macrophyte Control
L M H Dumping	L M H Marinas	L M H Poultry	L M H Commercial	P Water Level Fluctuations
L M H Roads	L M H Trash/Litter	L M H Feedlot	L M H Evidence of Fire	P Canada Geese Management
L M H Bridges/Causeways	L M H Surface Films, Scums, or Slicks	L M H Water Withdrawal	L M H Logging	P Other:
L M H Sewage Treatment	L M H Angling Pressure			* list chemical treatments if present:
L M H Schools	L M H Sports/Ballfields			_____

GENERAL LAKE INFORMATION

Hydrologic Lake Type: ☐ Reservoir (dam dependant) ☐ Drainage (natural, outlets present) ☐ Seepage (no outlets present)

Outlet Dams: ☐ None ☐ Artificial ☐ Natural

Motor Boat Density: ☐ High ☐ Low ☐ Restricted ☐ Banned

Swimability: ☐ Good ☐ Fair ☐ Not Swimmable

Swimming Beach Present?: ☐ Yes ☐ No

Lake Level Changes: ☐ Zero ☐ Elevation Change = _____ m

SHORELINE CHARACTERISTICS (% of shoreline within 20m)

Blank=Not Observed

Vegetation

Forest (trees and saplings)	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)
Grass (meadows, lawns etc.)	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)
Shrub	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)
Wetland	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)
Bare Ground (including beaches and paved areas)	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)

Anthropogenic

Agriculture	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)
Shoreline Mods (docks, riprap)	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)
Development (Residential & Urban)	<input type="radio"/> Rare (<5%)	<input type="radio"/> Sparse (5 to 25%)	<input type="radio"/> Moderate (25 to 75%)	<input type="radio"/> Extensive (>75%)

QUALITATIVE MACROPHYTE SURVEY

Blank=Not Observed

Emergent/Floating Coverage (% Lake Area)	<input type="radio"/> <5%	<input type="radio"/> 5 to 25%	<input type="radio"/> 25 to 75%	<input type="radio"/> >75%
Submergent Coverage (% Lake Area)	<input type="radio"/> <5%	<input type="radio"/> 5 to 25%	<input type="radio"/> 25 to 75%	<input type="radio"/> >75%
Macrophyte Density	<input type="radio"/> Absent	<input type="radio"/> Sparse	<input type="radio"/> Moderate	<input type="radio"/> High

WATERBODY CHARACTER

Pristine	<input type="radio"/> 5	<input type="radio"/> 4	<input type="radio"/> 3	<input type="radio"/> 2	<input type="radio"/> 1	Highly Disturbed
Appealing	<input type="radio"/> 5	<input type="radio"/> 4	<input type="radio"/> 3	<input type="radio"/> 2	<input type="radio"/> 1	Unappealing

LAKE ASSESSMENT FORM (cont.)

SITE ID: _____		DATE: ____/____/____	
QUALITATIVE ASSESSMENT OF ENVIRONMENTAL VALUE			
Ecological Integrity:		<input type="radio"/> Excellent	<input type="radio"/> Good
		<input type="radio"/> Fair	<input type="radio"/> Poor
General Assessment:			
Wildlife Observed:			
	Canada Geese: L M H Not observed		
Trophic State:		<input type="radio"/> Oligotrophic	<input type="radio"/> Mesotrophic
		<input type="radio"/> Eutrophic	<input type="radio"/> Hypereutrophic
Visual Assessment:			
Algal Abundance & Type:	If Bloom observed. Note location and extent. Digital photos recommended.		
	Bloom appearance(circle all that apply): a. "spilled paint" b. "pea soup" c. green streaks parallel to shore line d. large concentration of "green dots"		
Possible Nutrient Sources:			
Recreational Value:		<input type="radio"/> Excellent	<input type="radio"/> Good
		<input type="radio"/> Fair	<input type="radio"/> Poor
Conditions and Local Contacts:			
Observations (e.g. accessibility, boating, fishing, swimming, health concerns):			
Comments:			

APPENDIX F
Sample Collection Reference Guide
A. Harmful Algae Bloom (HAB) Sample Collection
B. Cyanotoxins in Raw Water

Sample collection to be submitted to BFBM for HABs follows the method for Phytoplankton Sampling, Chapter 6.10.1 of the NJDEP Field Sampling Procedures Manual, August 2005, <http://www.state.nj.us/dep/srp/guidance/fspm/>.

The following are examples of sample procedures. Sample container, holding time, and preservation should be verified with the lab used.

**A. Harmful Algae Bloom (HAB) Sample Collection
Division of Water Monitoring and Standards/
Bureau of Freshwater and Biological Monitoring (BFBM)**

Field Collection/ Analysis Procedures

OBJECTIVE

Harmful Algal Blooms, “HABs”, is the name given to the excessive growth, or “blooms”, of algae and algae-like bacteria which can be harmful to people and animals. These “blooms” often result in a thick coating or “mat” on the surface of a body of freshwater, generally in late-summer or early fall. Algae-like bacteria, often referred to as “cyanobacteria” or “blue-green algae” can form HABs that may produce chemicals which can be toxic to humans, pets, livestock or wildlife. These chemicals are called “cyanotoxins.”

Cyanotoxins can be produced by a wide variety of planktonic (i.e., free living in the water column) cyanobacteria. One of the most commonly occurring types are Microcystis species which can produce toxins called microcystins. Microcystins may cause adverse health effects to humans and animal if inhaled, ingested, or if contacted by skin or mucous membranes. Other types of cyanotoxins include anatoxin and cylindrospermopsin.

PROCEDURES

Equipment and Supplies

- Protective gloves
- Amber Glass 500 ml bottles –for BFBM lab analysis
- Cooler with ice.

Notifications

- Contact DEP Hotline to report incident and obtain a case number.
- Contact BFBM and provide case number.
- Complete HAB reporting form at www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. and submit to BFBM.
- BFBM will provide guidance regarding sample locations and sampling.
- BFBM will coordinate appropriate lab analysis.

Sample Collection/ Analysis/ Actions

- Protective gloves should be worn during sample collection and analysis. Avoid contact with water; if wading, boots should be worn.

Samples for BFBM analysis may include: cyanobacterial IDs, cell counts, toxin analyses (microcystins, anatoxin and/ or cylindrospermopsin) and/or chlorophyll a)

- Collect samples at designated locations, filling one (1) 500 ml amber glass bottle for lab analysis at BFBM.
- Samples should be collected just below the surface so mouth of bottle is immersed approximately 3-6 inches.
- With permanent marker, write lake name, date, and time on each bottle cap.
- Refrigerate samples, or place in cooler with ice.
- Contact BFBM to arrange for sample pickup/ delivery within 24 hours. Contact info below.
- Based on lab analysis, BFBM will recommend and coordinate advisories, and continued monitoring and analysis as needed

BFBM Contacts (609) 292-0427

Victor Poretti, Section Chief

Dean Bryson, Supervisor

Johannus Franken, Field Project Officer

Tom Miller, Lab Project Officer

Chris Kunz, Supervisor

Leslie McGeorge, Administrator

B. Cyanotoxins in Raw Water
Sample Collection Quick Reference Guide



Analyte	Collection/Storage Container	Preservation (at time of sampling)
Anatoxin-a	Amber glass <i>Avoid exposure to light, as this will degrade the toxin.</i>	Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of toxin. <i>Preservation is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation.</i> <i>Avoid exposure to high pH conditions, as this will degrade the toxin.</i>
BMAA	Clear glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS) <i>Avoid amber glass, as toxin will be lost due to adsorption to container surface.</i>	Freeze <i>Samples should be analyzed immediately or frozen to avoid degradation of toxin.</i>
Cylindrospermopsin	Clear or amber glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS)	None
Microcystins	Clear or amber glass Polyethylene terephthalate glycol (PETG) <i>Avoid all plastic containers other than PETG, as toxin will be lost due to adsorption to container surface.</i>	None
Saxitoxin	Clear or amber glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS)	Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of toxin. <i>Preservation is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation.</i>

Unless otherwise indicated, samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen. If samples are to be shipped, they should be shipped overnight, on ice.

R021516

APPENDIX G
Analysis Methods and Specifications
Field Strip Test
ELISA

Importance of Microcystins/Nodularins Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (of which there are many structural variants, or congeners) have been found in fresh water throughout the world. To date, approximately 80 variants of Microcystin have been isolated. The most common variant is Microcystin-LR. Other common Microcystin variants include YR, RR, and LW. These toxins are produced by many types of cyanobacteria (blue-green algae), including *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc*, *Anabaenopsis*, and terrestrial *Hapalosiphon*. Nodularins are produced by the genus *Nodularia* and they are found in marine and brackish water

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has led to death. Human and animal exposure to these toxins occurs most frequently through the ingestion of water, through drinking or during recreational activities in which water is swallowed. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, and therefore they may act as tumor promoters.

To protect consumers from adverse health effects caused by these toxins, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb ($\mu\text{g/L}$) in drinking water. For recreational bathing waters, the WHO has established the following guidelines:

- Relatively low risk of exposure effect at 4 ng/mL (ppb)
- Moderate probability of exposure effect at 20 ng/mL
- High probability of exposure effect – scums

The U.S. Environmental Protection Agency (EPA) has also established guidelines for Microcystins in drinking water:

- For children below school age, 0.3 $\mu\text{g/L}$ (ppb)
- For all other age groups, 1.6 $\mu\text{g/L}$ (ppb)

Performance Data

Test sensitivity:	The Abraxis Microcystins Strip Test for Recreational Water will detect Microcystins and Nodularins at 1 ng/mL or higher. At this level, the test line exhibits moderate intensity. At levels greater than 10 ng/mL the test line is not visible. When compared with samples of known Microcystins concentration, it is possible to obtain a semi-quantitative result.
Selectivity:	The assay exhibits very good cross-reactivity with all Microcystin cyclic peptide toxin congeners tested to date.
Cell Lysing:	When comparing samples lysed using the QuikLyse [™] reagents and the 3 cycle freeze/thaw method, average recovery obtained was 94%, SD = 16.7%.
Samples:	A sample correlation between the Abraxis Strip Test and ELISA methods showed a good correlation.

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R071216

Microcystins Strip Test

Immunochromatographic Strip Test for the Detection
of Microcystins and Nodularins in Recreational Water at 10 ppb



Product No. 520023 (5 Test), 520022 (20 Test)

1. General Description

The Abraxis Microcystins Strip Test for Recreational Water is a rapid immunochromatographic test, designed solely for the use in the qualitative screening of Microcystins and Nodularins in recreational water (freshwater samples only; please see the Brackish or Sea Water Sample Preparation technical bulletin for information on the screening of marine water samples). A rapid cell lysis step (QuikLyse[™]) performed prior to testing is required to measure total Microcystins (dissolved, or free, plus cell-bound). The Abraxis Microcystins Strip Test provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods.

* Patent Pending

2. Safety Instructions

Discard samples according to local, state and federal regulations.

3. Storage and Stability

The Microcystins Strip Kit should be stored between 4–30°C. The test strips, test vials and water samples to be analyzed should be at room temperature before use.

4. Test Principle

The test is based on the recognition of Microcystins, Nodularins, and their congeners by specific antibodies. The toxin conjugate competes for antibody binding sites with Microcystins/Nodularins that may be present in the water sample. The test device consists of a vial containing specific antibodies for Microcystins and Nodularins labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of Microcystins in the water sample and, therefore, should be present in all reactions.

In the absence of toxin in the water sample, the colloidal gold labeled antibody complex moves with the water sample by capillary action to contact the immobilized Microcystins conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area. The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect the toxin at or above the cut-off point established for the toxin. If Microcystins are present in the water sample, they compete with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of toxin is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate, therefore preventing the development of a colored line. If a colored line is not visible in the test line region, or if the test line is lighter than the control line, Microcystins are present at a level > 2.5 ppb. Semi-quantitative results in the range of 0-10 ppb can be obtained by comparing the sample test strip appearance to the appearance of test strips from solutions of known Microcystins concentrations (control solutions). Microcystins controls are available through Abraxis (PN 422011).

5. Limitations of the Microcystins Strip Test, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors include:

Inadequate storage conditions of the test strip, too long or too short incubation times, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The test is designed for use with freshwater recreational waters. The use of the test with brackish or seawater samples will produce inaccurate results. Please see the Brackish or Sea Water Sample Preparation technical bulletin for information on the preparation and screening of marine water samples using the Microcystins Strip Test for Finished Drinking Water. The Microcystins Strip Test provides only a preliminary qualitative test result. Use another more quantitative analytical method such as ELISA or instrumental analysis to obtain a confirmed quantitative analytical result. Apply good judgement to any test result, particularly when preliminary positive results are observed.

6. Warnings and Precautions

-The Microcystins Strip Test for Recreational Water is for the screening of freshwater recreational water samples for total Microcystins (free and cell-bound). Please see the Brackish or Sea Water Sample Preparation technical bulletin for the preparation and screening of marine water samples using the Microcystins Strip Test for Finished Drinking Water.

-Use of the Microcystins Test Strips **without** the QuikLyse™ reagents will adversely affect the performance of the test, producing inaccurate results. To test samples without using QuikLyse™ reagents for cell lysis, such as when testing for free Microcystins only or when testing samples which have been previously lysed (such as those which have undergone the freeze/thaw method), please use the Abraxis Microcystins Strip Test for Finished Drinking Water at 1 ppb, PN 520016 (5 Test) or PN 520017 (20 Test).

-Use only the Microcystins test strips and QuikLyse™ reagents from one kit lot, as they have been adjusted in combination.

-All reagents and samples should be allowed to reach room temperature before testing.

-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.

-For test strips packaged in a desiccated vial, the vial should be kept completely closed except for opening to remove test strips. When re-closing, snap lid firmly.

-Avoid cross-contamination of water samples by using a new sample vial and disposable pipette for each sample.

-Samples containing unusually large amounts of algal blooms or very thick algal scums should be diluted 1:1 with deionized or distilled water prior to lysis, as overly viscous samples may not allow for uniform cell lysis or proper capillary flow up the test strip. Diluted samples will have a cut-off of 20 ppb.

-Use reasonable judgment when interpreting the test results.

-Results should be interpreted within 5-10 minutes after completion of the test.

7. Sample Collection and Handling

-Collect water samples in glass or polyethylene terephthalate (PETG) containers only. The use of other types of plastic containers may result in adsorptive loss of Microcystins, producing inaccurate (falsely low) results.

-Samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen.

A. Materials Provided

1. Microcystins test strips in a desiccated container
2. Sample collection vials
3. Lysis vials
4. Graduated disposable pipettes (calibrated at 1 mL)
5. Forceps
6. Reagent papers
7. Conical test vials
8. Disposable transfer pipettes
9. User's guide

B. Additional Materials (not provided with the test)

1. Timer
2. Microcystins Check Samples, Abraxis PN 422011, for the preparation of control solutions which can be analyzed with samples, to obtain semi-quantitative sample results (see section C, Assay Controls, below).

C. Controls

It is a good laboratory practice to use positive and negative controls to ensure proper test performance. Water samples containing known quantities of Microcystins (positive and negative controls) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected.

D. Test Preparation

1. Allow the reagents and water sample to reach room temperature before use.
2. Remove the number of test strips required from the package. The remaining strips are stored in the tightly closed desiccated container.

E. Procedure

When analyzing for total Microcystins (dissolved, or free, and cell-bound), which may be present in recreational waters, a sample lysis is necessary before analysis. The Abraxis QuikLyse™ reagents provide a rapid option for cell lysis.

1. Using a new graduated disposable pipette for each sample, draw the sample to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of sample to the lysis vial.
2. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes, to begin the cell lysis.
3. Using the forceps provided, add 1 reagent paper to the lysis vial.

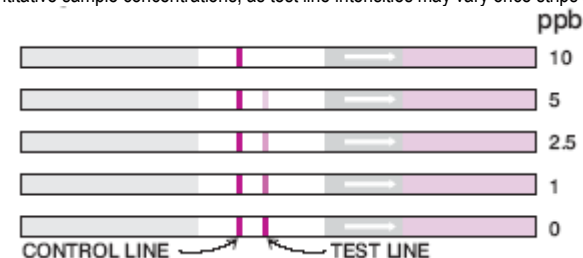
4. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes.
5. Label conical test vials for each sample to be tested.
6. Using a new disposable transfer pipette for each sample, transfer 7 drops (approximately 200 µL) of the previously lysed water sample (Steps 1-4 above) to the appropriately labeled conical test vial.
7. Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are completely dissolved (dried reagents will dissolve, turning the sample purple).
8. Insert test strip (arrows down) into the conical vial.
9. Allow the test to develop for 10 minutes.
10. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
11. Read the results visually, as explained below in section F, Interpretation of Results.

F. Interpretation of Results

Sample concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the control line indicates a result which is below the limit of detection of the test. Test strips with a test line which is lighter than the control line indicates a result which is <10 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 10 ppb. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

<u>Control Line</u>	<u>Test Line</u>	<u>Interpretation</u>
No control line present	No test line present	Invalid result
Control line present	No test line present	>10 ng/mL (ppb)
Control line present	Moderate to equal intensity test line present	Between 0 and 10 ng/mL (ppb)

The appearance of test strips may also be compared to the illustration below to determine approximate sample concentration ranges. Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents. To obtain semi-quantitative results in the range of 0-10 ppb, solutions of known Microcystins concentration (control solutions) must be tested concurrently with samples. Sample test line intensities can then be compared with control solution test line intensities, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.



Alternately, test strips can also be interpreted using the AbraScan test strip reader (PN 475025), which provides objective determination of line intensities for consistent interpretation of results as well as a digital photographic record of all test strips.

G. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods. These services are available from commercial analytical laboratories such as Green Water Labs (www.greenwaterlab.com).

H. References

- (1) W. J. Fischer, I. Garthwaite, C.O. Miles, K.M. Ross, J.B. Aggen, A.R. Chamberlain, N.A. Towers, and D.R. Dietrich, Congener-Independent Immunoassay for Microcystins and Nodularins. Environ. Sci. Technol. 35, 2002, 4849-4858.
- (2) Worldwide Patenting PCT WO 01/18059 A2.
- (3) U.S. Patent Number 6,967,240.

Importance of Microcystins/Nodularins Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (of which there are many structural variants, or congeners) have been found in fresh water throughout the world. To date, approximately 80 variants of Microcystin have been isolated. The most common variant is Microcystin-LR. Other common Microcystin variants include YR, RR, and LW. These toxins are produced by many types of cyanobacteria (blue-green algae), including *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc*, *Anabaenopsis*, and terrestrial *Hapalosiphon*. Nodularins are produced by the genus *Nodularia* and are found in marine and brackish water.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has led to death. Human and animal exposure to these toxins occurs most frequently through ingestion of water, through drinking or during recreational activities in which water is swallowed. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, and therefore may act as tumor promoters.

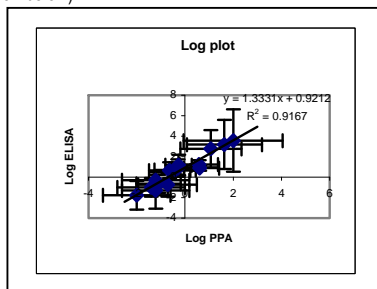
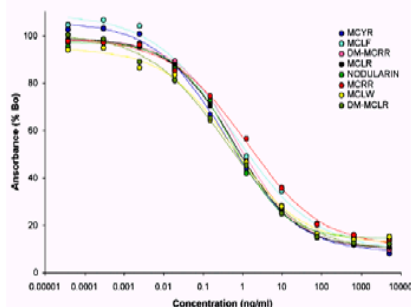
To protect consumers from adverse health effects caused by these toxins, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water.

Performance Data

Test sensitivity: The detection limit for this assay, based on MC-LR, is 0.10 ppb (µg/L).

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for samples: <15%.

Selectivity*: The assay exhibits very good cross-reactivity with all cyanobacterial cyclic peptide toxin congeners tested to date (see cross-reactivity illustration below).



Samples: Sample correlation between HPLC, PPA, and ELISA methods showed a good correlation (see ELISA and PPA correlation above).

References

- (1) W. J. Fischer, I. Garthwaite, C.O. Miles, K.M. Ross, J.B. Aggen, A.R. Chamberlin, N.A. Towers, and D.R. Dietrich, Congener-Independent Immunoassay for Microcystins and Nodularins. *Environ. Sci. Technol.* 35, 2001, 4849-4858.
- (2) Worldwide Patenting PCT WO 01/18059 A2.
- (3) U.S. Patent Number 6,967,240.

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R011315QH

Microcystins-ADDA ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Congener-Independent* Determination of Microcystins and Nodularins in Water Samples



Product No. 520011OH

1. General Description

The Abraxis Microcystins-ADDA ELISA is an immunoassay for the quantitative and sensitive congener-independent* detection of Microcystins and Nodularins in water samples. This test is suitable for the quantitative and/or qualitative detection of Microcystins and Nodularins in water samples [please refer to the appropriate technical bulletins for sample collection, handling, and treatment of drinking (treated and untreated) and recreational water samples]. If necessary, positive samples can be confirmed by HPLC, protein phosphatase assay, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Microcystins. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The Microcystins-ADDA ELISA kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is an indirect competitive ELISA for the congener-independent detection of Microcystins and Nodularins. It is based on the recognition of Microcystins, Nodularins, and their congeners by specific antibodies. Toxin, when present in a sample, and a Microcystins-protein analogue immobilized on the plate compete for the binding sites of the anti-Microcystins/Nodularins antibodies in solution. The plate is then washed and a second antibody-HRP label is added. After a second washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Microcystins-ADDA ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

Samples containing methanol must be diluted to a concentration < 5% methanol to avoid matrix effects.

Seawater samples must be diluted to a concentration ≤ 2.5% to avoid matrix effects. Alternately, if a lower detection limit is required, interfering compounds can be removed from seawater or brackish water samples prior to analysis. Please see the Microcystins in Brackish Water or Seawater Sample Preparation for the Microcystins-ADDA ELISA Technical Bulletin (available upon request).

No matrix effects have been observed with samples which have been treated with sodium thiosulfate at concentrations ≤ 1 mg/mL or ascorbic acid at concentrations ≤ 1 mg/mL.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Materials Provided

1. Microtiter plate (12 X 8 strips) coated with an analog of Microcystins conjugated to a protein
2. Standards (6): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb
3. Control: 0.75 ± 0.185 ppb, prepared from a secondary source, for use as a Quality Control Standard (QCS)
4. Low Calibration Range Check (LCRC): 0.40 ± 0.16 ppb
5. Sample Diluent, for use as a Laboratory Reagent Blank (LRB) and for dilution of samples above the range of the standard curve
6. Antibody Solution
7. Anti-Sheep-HRP Conjugate Solution
8. Wash Solution (5X) Concentrate, must be diluted prior to use, see Test Preparation (Section E)
9. Substrate (Color) Solution (TMB)
10. Stop Solution

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-200 μ L)
2. Multi-channel pipette (50-300 μ L), stepper pipette (50-300 μ L), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for 1X diluted wash solution, see Test Preparation, Section E)
5. Graduated cylinder
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm
9. Microtiter plate reader (wavelength 450 nm)
10. Microtiter plate washer (optional)

C. Sample Collection and Handling

Collect water samples in glass or PETG containers and test within 24 hours. Use of other types of plastic collection and/or storage containers may result in adsorptive loss of Microcystins, producing inaccurate (falsely low) results. Drinking water samples should be treated with sodium thiosulfate immediately after collection (refer to appropriate technical bulletin). If samples must be held for longer periods (up to 5 days), samples should be stored refrigerated. For storage periods greater than 5 days, samples should be stored frozen.

If total Microcystins concentration (free and cell bound) is required, an appropriate cell lysing procedure (freeze and thaw, QuikLyse™, etc.) must be performed prior to analysis. *Note: The use of sonication in cell lysing can negatively affect toxin concentrations, producing falsely low sample results. Please see the appropriate sample preparation technical bulletin for additional information on cell lysis.*

Samples may be filtered prior to analysis using glass fiber filters (Environmental Express 1.2 μ m syringe filters (Environmental Express part number SF012G) are recommended). If determining total Microcystins concentration, samples should be lysed prior to filtration to prevent the removal of cell-bound Microcystins, which would cause inaccurate (falsely low) results. *Note: The use of alternate filter types (non-glass fiber filters) may produce falsely low sample results, as Microcystins may bind to the filter material, removing it from the sample.*

D. Notes and Precautions

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary.

The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for the addition of the antibody, enzyme conjugate, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, conjugate, color, and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Please use only the reagents and standards from one kit lot in one test, as they have been adjusted in combination.

E. Test Preparation

1. Allow the reagents and samples to reach ambient temperature before use.
2. Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly sealed).
3. The standards, control, low calibration range check (LCRC), sample diluent (LRB), antibody, enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 6	LRB									
B	Std 0	Std 6	LRB									
C	Std 1	Std 5	Sample1									
D	Std 1	Std 5	Sample1									
E	Std 2	Control	Sample2									
F	Std 2	Control	Sample2									
G	Std 3	LCRC	etc.									
H	Std 3	LCRC	etc.									

Std 0-Std5: Standards

Contr.: Control (QCS)

LCRC: Low Calibration Range Check

LRB: Laboratory Reagent Blank

Samp1, Samp2, etc: Samples

G. Assay Procedure

1. Add 50 μ L of the standard solutions, control, LCRC, LRB, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50 μ L of the antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 90 minutes at room temperature.
3. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the 1X wash buffer solution. Please use at least a volume of 250 μ L of wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
4. Add 100 μ L of the enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 30 minutes at room temperature.
5. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the 1X wash buffer solution. Please use at least a volume of 250 μ L of wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 μ L of substrate (color) solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from sunlight.
7. Add 50 μ L of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B₀ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B₀ for each standard on the vertical linear (y) axis versus the corresponding Microcystins concentration on the horizontal logarithmic (x) axis on graph paper. %B/B₀ for the control (QCS), LCRC, LRB, and samples will then yield levels in ppb of Microcystins by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Microcystins than standard 1 (0.15 ppb) should be reported as containing < 0.15 ppb of Microcystins. Samples showing a higher concentration than standard 5 (5.0 ppb) must be diluted to obtain accurate results. The concentration of the positive control (QCS) provided should be 0.75 ± 0.185 ppb; the LCRC should be 0.40 ± 0.16 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrators. Samples with lower absorbances than a calibrator will have concentrations of Microcystins greater than that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of Microcystins less than that calibrator.

Importance of Cylindrospermopsin Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Cylindrospermopsin is a toxin produced by several different strains of cyanobacteria (blue-green algae) and has been found in fresh water throughout the world. Certain strains of *Cylindrospermopsis raciborskii* (found in Australia, Hungary, and the United States), *Umezakia natans* (found in Japan), and *Aphanizomenon ovalisporum* (found in Australia and Israel) have been found to produce Cylindrospermopsin. The production of Cylindrospermopsin seems to be strain specific rather than species specific.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has led to death. Human exposure to Cylindrospermopsin can occur through ingestion of contaminated water or food (fish) or during recreational activities in which water is swallowed. Dermal contact with Cylindrospermopsin may occur during showering or bathing, or during recreational activities such as swimming or boating. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of protein synthesis and glutathione, leading to cell death.

To protect against adverse health effects, the U.S. Environmental Protection Agency (EPA) has established guidelines for Cylindrospermopsin in drinking water:

- For children pre-school age and younger (less than six years old), 0.7 µg/L (ppb)
- For school-age children and adults, 3.0 µg/L (ppb)

Performance Data

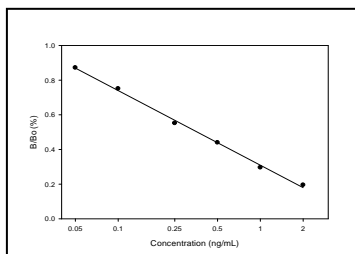
Test sensitivity: The detection limit for this assay is 0.040 ppb (µg/L).

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for samples: <15%.

Specificity: This ELISA recognizes Cylindrospermopsin and related compounds with varying degrees:

Cylindrospermopsin	100%
Deoxy-Cylindrospermopsin	112%

Standard Curve:



Samples: A sample correlation between the ELISA and HPLC methods showed a good correlation.

Recovery

Four (4) groundwater samples were spiked with various levels of Cylindrospermopsin and assayed using the Abraxis Cylindrospermopsin Assay:

Spike Level (ppb)	Mean (ppb)	Std.Dev. (ppb)	Recovery (%)
0.1	0.101	0.019	101
0.25	0.269	0.026	108
0.5	0.514	0.038	103
1.0	0.982	0.113	98
Average			103

Precision

Control	1	2	3
Replicates	3	3	3
Days	3	3	3
n	9	9	9
Mean (ppb)	0.198	0.501	1.01
% CV (within assay)	6.2	4.3	5.2
% CV (between assay)	8.3	5.3	4.9

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis LLC makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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R090415

Cylindrospermopsin ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of
Cylindrospermopsin in Water Samples



Product No. 522011

1. General Description

The Abraxis Cylindrospermopsin ELISA is an immunoassay for the quantitative and sensitive detection of Cylindrospermopsin in water samples. No additional sample preparation is required prior to analysis. If necessary, positive samples can be confirmed by HPLC or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Cylindrospermopsin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The Cylindrospermopsin ELISA kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA for the detection of Cylindrospermopsin. It is based on the recognition of Cylindrospermopsin by specific antibodies. Cylindrospermopsin, when present in a sample, and a Cylindrospermopsin-HRP analogue compete for the binding sites of rabbit anti-Cylindrospermopsin antibodies in solution. The anti-Cylindrospermopsin antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Cylindrospermopsin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Cylindrospermopsin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the Cylindrospermopsin assay results: aluminum oxide, calcium chloride, calcium sulfate, manganese sulfate, magnesium sulfate, magnesium chlorides, sodium chloride, potassium phosphate, and sodium thiosulfate up to 10,000 ppm; sodium nitrate and zinc sulfate up to 1,000 ppm; humic acid and ferric sulfate up to 100 ppm; copper chloride up to 10 ppm; Lugol's solution up to 0.01%.

Samples containing methanol must be diluted to a concentration ≤ 20% methanol to avoid matrix effects.

Seawater samples must also be diluted to a concentration ≤ 20% to avoid matrix effects. Alternately, if a lower detection limit is required, interfering compounds can be removed from seawater or brackish water samples prior to analysis. Please see the Cylindrospermopsin in Brackish Water or Seawater Sample Preparation Technical Bulletin (available upon request).

No matrix effects have been observed with samples which have been treated with sodium thiosulfate at concentrations up to and including 1 mg/mL. Please see Sample Collection and Handling (Section C) for additional information on sample collection, preservation, and storage.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a second antibody (goat anti-rabbit)
2. Standards (7): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ppb
3. Control: 0.75 ± 0.15 ppb, prepared from a secondary source, for use as a Quality Control Standard (QCS)
4. Sample Diluent, for use as a Laboratory Reagent Blank (LRB) and for dilution of samples above the range of the standard curve
5. Cyindrospermopsin-HRP Conjugate Solution
6. Antibody Solution (rabbit anti-Cyindrospermopsin)
7. Wash Solution (5X) Concentrate, must be diluted before use, see Test Preparation (Section E)
8. Substrate (Color) Solution (TMB)
9. Stop Solution

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-200 μ L)
2. Multi-channel pipette (50-300 μ L), stepper pipette (50-300 μ L), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for 1X diluted wash solution, see Test Preparation, Section E)
5. Graduated cylinder
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm
9. Microtiter plate reader (wavelength 450)
10. Microtiter plate washer (optional)

C. Sample Collection and Handling

Water samples should be collected in glass, polyethylene terephthalate glycol (PETG), high density polyethylene (HDPE), polycarbonate (PC), polypropylene (PP), or polystyrene (PS) containers. Samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen.

Finished (treated) drinking water samples must be preserved (quenched) with sodium thiosulfate immediately after collection to remove residual chlorine. Samples can be quenched with sodium thiosulfate at concentrations up to and including 1 mg/mL. The quenching of residual chlorine is necessary for treated water samples only. Raw (untreated) drinking water samples (samples not treated with chlorine) do not require additional reagents at the time of collection.

D. Notes and Precautions

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary.

The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for the addition of the antibody, enzyme conjugate, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, conjugate, color, and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

E. Test Preparation

1. Allow the reagents and samples to reach ambient temperature before use.
2. Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly closed).
3. The standards, control, sample diluent (LRB), antibody, enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4 .

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	LRB									
B	Std 0	Std 4	LRB									
C	Std 1	Std 5	Samp1									
D	Std 1	Std 5	Samp1									
E	Std 2	Std 6	Samp2									
F	Std 2	Std 6	Samp2									
G	Std 3	Contr.	etc.									
H	Std 3	Contr.	etc.									

Sd0-Sd6: Standards

Contr.: Control (QCS)

LRB: Laboratory Reagent Blank

Samp1, Samp2, etc.: Samples

G. Assay Procedure

1. Add **50 μ L of the standard solutions, control (QCS), LRB, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50 μ L of the enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50 μ L of the antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **45 minutes** at room temperature.
4. Remove the covering and decant the contents of the wells into a sink. Wash the strips **four times** using the 1X wash buffer solution. Please use at least a volume of **250 μ L of wash buffer** for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add **100 μ L of substrate (color) solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **30-45 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100 μ L of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Cyindrospermopsin concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control (QCS), LRB, and samples will then yield levels in ppb of Cyindrospermopsin by interpolation using the standard curve. Results can also be determined by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Cyindrospermopsin than standard 1 (0.05 ppb) should be reported as containing < 0.05 ppb of Cyindrospermopsin. Samples showing a higher concentration than standard 6 (2.0 ppb) must be diluted to obtain accurate results. The concentration of the positive control (QCS) provided should be 0.75 ± 0.15 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrators. Samples with lower absorbances than a calibrator will have concentrations of Cyindrospermopsin greater than that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of Cyindrospermopsin less than that calibrator.

I. References

- (1) Cyindrospermopsin, Review of Toxicological Literature. Prepared by Integrated Laboratory Systems for Scott Masten, National Institute of Health Sciences, RTP, NC. Contract Number N01-ES-65402, December 2000.

Importance of Anatoxin-a Determination

Anatoxin-a is an alkaloid neurotoxin produced by some species of cyanobacteria (blue-green algae). It is one of the most toxic of the cyanobacterial toxins. In humans and other animals, the skeletal neuromuscular junction constitutes a primary target for Anatoxin-a (Anatoxin-a can also cross the blood-brain barrier). The neuromuscular junction is specialized for the rapid transmission of neuronal information from the pre-synaptic nerve terminal to the post-synaptic muscle fiber. This transmission is mediated by the synchronous release of the neurotransmitter acetylcholine (ACh), which activates nicotinic acetylcholine receptors (nAChRs) in the muscle endplate, triggering a series of events that lead to muscle contraction. Most ACh molecules are hydrolyzed by acetylcholinesterases, which are highly concentrated at the neuromuscular junction. Anatoxin-a functions as an agonist of nAChRs, like ACh, but is about 20 times more potent. Unlike ACh, it is not degraded by acetylcholinesterases and produces sustained depolarization of the muscle endplate, causing over stimulation of the muscles, leading to muscle fatigue and ultimately paralysis. Symptoms begin within 5 minutes of ingestion of Anatoxin-a and progress rapidly, resulting in cyanosis, convulsions, cardiac arrhythmia, and respiratory paralysis, which ultimately results in death due to suffocation.

Humans and other animals may be exposed to Anatoxin-a through ingestion of contaminated water, through drinking or during recreational activities in which water is swallowed. Due to the potential for serious harm and even death, many countries are expanding monitoring programs to include Anatoxin-a and are establishing regulations regarding the amount of Anatoxin-a in drinking and recreational waters. New Zealand is among those taking regulatory action, establishing a 6.0 µg/L provisional maximum acceptable value (MAV) for Anatoxin-a.

The Abraxis Anatoxin-a ELISA Assay can be performed in less than 90 minutes. Only a few milliliters of sample are required.

Performance Data

Test sensitivity: The detection limit, based on Anatoxin-a, (90% B/B₀) is approximately 0.1 ppb (µg/L). The middle of the test (50% B/B₀) is approximately 1.38 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

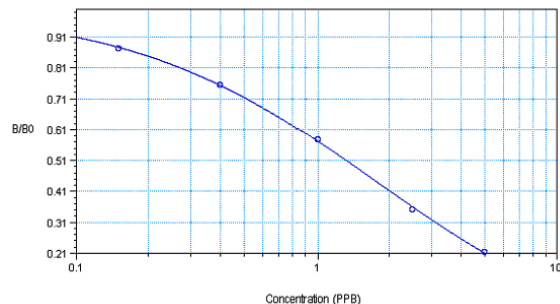
Test reproducibility: Intra and inter assay: < 10%

Recoveries:	Level (ppb)	% Recovery
	0.25	103.3
	0.50	98.0
	1.50	104.4
	3.00	103.1

Specificity: Cross-reactivity of the Abraxis Anatoxin-a Plate Kit for various congeners:

(+)Anatoxin-a	100.0%
Homoanatoxin-a	124.8%
(-)Anatoxin-a	0.3%

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

General Limited Warranty: Abraxis, Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

*The monoclonal antibody and enzyme conjugate included in the Abraxis Anatoxin-a ELISA have been licensed (Patent Application P201531661) from the Spanish National Research Council (CSIC) and the University of Valencia (UEVG).

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R053116

Anatoxin-a ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of
Anatoxin-a* in Water Samples
Product No. 520060



1. General Description

The Abraxis Anatoxin-a ELISA Plate Kit is an immunoassay for the quantitative and sensitive screening of Anatoxin-a in water samples. This test is suitable for the quantitative and/or qualitative screening of Anatoxin-a in drinking and recreational water samples (please refer to Sample Collection and Handling, section C). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Anatoxin-a. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of these solutions with skin and mucous membranes. If these reagents come in contact with skin, wash thoroughly with water.

3. Storage and Stability

The Anatoxin-a ELISA Kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Anatoxin-a by a monoclonal antibody. Anatoxin-a, when present in a sample, and an Anatoxin-a-enzyme conjugate compete for the binding sites of mouse anti-Anatoxin-a antibodies in solution. The Anatoxin-a antibodies are then bound by a second antibody (anti-mouse) immobilized on the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Anatoxin-a present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Anatoxin-a ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

Immediately upon collection, fresh water samples must be preserved with the provided Sample Diluent (10X) Concentrate to prevent degradation of Anatoxin-a (please refer to Sample Collection and Handling, section C).

Anatoxin-a will degrade when exposed to natural and artificial light and/or high pH conditions. Samples that have been exposed to natural or artificial light and/or treated with reagents that raise the natural sample pH may produce results that are falsely low. Samples should be adjusted to between pH 5 and pH 7 and protected from light.

Samples containing methanol must be diluted to a concentration < 2.5% methanol to avoid matrix effects.

Seawater samples up to 37 parts per thousand were tested and no matrix effects were detected. Average recovery of spiked seawater samples was 104%.

Anatoxin-a is an intracellular, as well as extracellular, toxin. Therefore, to measure total Anatoxin-a, cell lysing will be required. Once the sample is preserved, three freeze/thaw cycles are recommended for cell lysing.

No matrix effects have been observed with samples that have been treated with ascorbic acid at concentrations ≤ 1 mg/mL. **Sodium thiosulfate should not be used to treat samples, as sodium thiosulfate will degrade Anatoxin-a, producing inaccurate (falsely low) results.**

Mistakes in handling the test can also cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, exposure to direct or indirect sunlight during the substrate reaction, or extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate coated with a secondary antibody (anti-mouse), in a resealable aluminum pouch
2. Lyophilized Anatoxin-a-HRP Enzyme Conjugate, 3 vials
3. Conjugate Diluent, 12 mL
4. Lyophilized Anti-Anatoxin-a Antibody, 3 vials
5. Antibody Diluent, 12 mL
6. Empty clear and amber HDPE bottles for combining reconstituted Enzyme Conjugate and Antibody (if necessary)
7. (+)Anatoxin-a Standards (6): 0, 0.15, 0.40, 1.0, 2.5, 5.0 ppb, 1 mL each
8. Control at 0.75 ± 0.185 ppb, 1 mL
9. Sample Diluent (10X) Concentrate, 2 X 25 mL
10. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
11. Color (Substrate) Solution (TMB), 12 mL
12. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 μ L)
2. Multi-channel pipette (10-300 μ L), stepper pipette (10-300 μ L), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-300 μ L)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Deionized or distilled water
6. Container with 500 mL capacity (for diluted 1X Wash Solution, see Test Preparation, Section D)
7. Paper towels or equivalent absorbent material
8. Timer

C. Sample Collection and Handling

Collect water samples in amber glass sample containers. Drinking water samples should be treated with ascorbic acid (up to 1 mg/mL) immediately after collection to remove residual chlorine. *Do not use sodium thiosulfate. Sodium thiosulfate will degrade Anatoxin-a.*

Immediately upon collection, fresh water samples must be preserved using the Sample Diluent (10X) Concentrate (1 mL of 10X Sample Diluent Concentrate per 9 mL of water sample), to prevent degradation of Anatoxin-a. Samples should be adjusted to between pH 5 and pH 7 and protected from exposure to natural and artificial light, as exposure to light and/or high pH will cause degradation of Anatoxin-a. Store samples refrigerated (up to 28 days). For storage periods greater than 28 days, samples should be stored frozen. Seawater samples do not need to be preserved but the same pH and storage conditions should be applied.

Anatoxin-a is an intracellular, as well as extracellular, toxin. Therefore, to measure total Anatoxin-a, cell lysing will be required. Once the sample is preserved, three freeze/thaw cycles are recommended for cell lysing. This procedure using the three freeze/thaw cycles will not degrade Anatoxin-a.

Preserved fresh water or seawater samples may be filtered following cell lysing and prior to analysis using any of the following syringe filters: Environmental Express 0.2 mm PES (PN SF020E), Pall Acrodisc® 0.2 mm PVDF (PN 4450), Supor® membrane syringe filters (PN 4612), or Environmental Express 1.2 mm Glass Fiber (PN SF012G). *Note: Fresh water samples must be preserved (and lysed) prior to filtration or Anatoxin-a may bind to the filter, removing it from the sample, and producing falsely low sample results.*

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods across the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Allow the microtiter plate, reagents, and samples to reach room temperature before use.
2. The enzyme conjugate and antibody need to be reconstituted prior to use. Add 3 mL of the appropriate diluent to the appropriate vial and vortex well. Let sit for at least 10 minutes and re-vortex prior to use. **If more than one vial is required for testing, combine the reconstituted enzyme conjugate vials in the amber HDPE bottle and the reconstituted antibody vials in the clear HDPE bottle prior to use.** The solutions are stable for up to 2 weeks if stored at 4-8°C and up to 1 month if stored frozen.
3. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed.
4. The standard solutions, substrate and stop solutions are ready to use and do not require any further dilutions.
5. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water.
6. Dilute the Sample Diluent (10X) Concentrate at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of Sample Diluent (10X) Concentrate into 9 mL of deionized water) as needed for sample dilutions.
7. The stop solution must be handled with care as it contains diluted H_2SO_4 .
8. After analysis, store the remaining kit components in the refrigerator (4-8°C).

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Contr.										
F	Std 2	Contr.										
G	Std 3	Samp1										
H	Std 3	Samp1										

Std 0-Std5: Standards

Contr.: Control

Samp1, Samp2, etc: Samples

F. Assay Procedure

1. Add **50 μ L of the standard solutions, control, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50 μ L of the reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50 μ L of the reconstituted antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for **60 minutes** at room temperature.
4. Remove the covering and decant the contents of the wells into a sink. Wash the strips **four times** using the 1X wash buffer solution. Please use at least a volume of **250 μ L of wash buffer** for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add **100 μ L of substrate (color) solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **20-30 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100 μ L of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Anatoxin-a concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control and samples will then yield levels in ppb of Anatoxin-a by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

Results for fresh water samples which have been preserved with Sample Diluent (10X) Concentrate as described in Sample Collection and Handling (section C) must be multiplied by a factor of 1.1 to account for the initial dilution.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Anatoxin-a than standard 1 (0.15 ppb) should be reported as containing < 0.15 ppb of Anatoxin-a (< 0.165 ppb for preserved water samples). Samples showing a higher concentration than standard 5 (5.0 ppb) should be reported as containing > 5.0 ppb of Anatoxin-a (> 5.5 ppb for preserved water samples) or must be diluted using 1X Sample Diluent and re-analyzed to obtain accurate results. The concentration of the positive control provided should be 0.75 ± 0.185 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrators. Samples with lower absorbances than a calibrator will have concentrations of Anatoxin-a greater than that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of Anatoxin-a less than that calibrator.

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

APPENDIX H

World Health Organization (WHO) Guidance

New Jersey has 2017 guidance for cyanoHABs as per DSREH Appendix and this Strategy, and EPA has draft recreational cyanotoxin values (2016): the 2009 WHO guidance is provided here for informational purposes.

For recreational waters, the World Health Organization (WHO) concludes that a single guideline value for cyanobacteria or cyanotoxins is not appropriate. Due to the variety of possible exposures through recreational activities (contact, ingestion and inhalation) it is necessary to differentiate between the chiefly irritative symptoms caused by unknown cyanobacterial substances and the more severe health effects due to exposure to high concentrations of known cyanotoxins, particularly microcystins. The WHO guidance values for the relative probability of acute health effects during recreational exposure to cyanobacteria and microcystins are:

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

World Health Organization. Guidelines for Safe Recreational Water Environments. 2009.
http://www.who.int/water_sanitation_health/publications/srwe1/en/.

APPENDIX I

DSREH Document: *Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin –A*

RECOMMENDED NJ ACTION LEVEL AND HEALTH ADVISORY GUIDELINES FOR RECREATIONAL EXPOSURE TO MICROCYSTIN-LR, CYLINDROSPERMOPSIN, AND ANATOXIN-A

Thomas Atherholt, Alan Stern, and Gloria Post
NJDEP Division of Science, Research and Environmental Health
April 6, 2017 (Updated: July 6, 2017)

Introduction

Harmful algal bloom events (HABs) result from excessive growth of cyanobacteria (i.e. photosynthetic bacteria also known as “blue-green algae”) in waterbodies. HABs are ephemeral in nature. Although certain environmental conditions are known to favor the development of a HAB (sunlight, high concentrations of nutrients, stagnant water, warm temperatures), scientists have not been able to determine a method that can accurately predict when a HAB event will occur. In addition, the location within a waterbody where a HAB occurs often depends on the prevailing wind direction and/or currents. Some species of cyanobacteria can produce chemicals (cyanotoxins) that are toxic to humans and animals if sufficient exposure occurs. However, cyanotoxins are not always produced during a cyanobacteria bloom event, and when they are produced, the comparative amount produced can be low or high. Over a hundred different cyanotoxins (including “variants” within a toxin “family”) have been identified. The specific toxins involved and their respective amounts can differ from one HAB event to another.

Therefore, it is difficult at best to accurately quantify human health risk during recreational exposure to a HAB event. Despite this difficulty, USEPA (2016) developed draft recreational criteria/swimming advisories for two cyanotoxins, while a number of states, as well as the World Health Organization (WHO), have derived their own “action levels” or health advisory guidelines based on cyanobacteria cell counts and/or concentrations of a few of the more toxic, commonly-occurring cyanotoxins.

The Bureau of Freshwater and Biological Monitoring (BFBM) of the NJ Department of Environmental Protection has developed the laboratory capability to measure levels of three of the most toxic, commonly observed cyanobacterial toxins in freshwater lakes, namely microcystins, cylindrospermopsin, and anatoxin-a. The first two are hepatic (liver) toxins while anatoxin-a is a neurotoxin. The methods are provided in [Appendix 1](#).

There are two general types of health effects concerns for recreational exposures to toxins from blue green algae/cyanobacteria:

¹ In May 2015, the USEPA provided guidance values for two cyanotoxins, microcystin-LR and cylindrospermopsin, for drinking water exposure. See USEPA (2015a) and USEPA (2015b).

1. Allergic and irritation reactions which occur from dermal contact with cyanobacterial substances in general (not specifically cyanotoxins) in some individuals at cyanobacteria levels below those which are of concern for liver toxicity.
2. Higher exposure levels with potential for liver or neurological toxicity.

The Division of Science, Research and Environmental Health (DSREH) was asked by BFBM to recommend action levels for recreational exposure to HABs, including health advisory guidance values for the three cyanotoxins mentioned above, based on review of the cyanotoxin guidance values developed by the WHO and by various states, and other relevant information. WHO recommendations are discussed in [Appendix 2](#), and recommendations of other states are presented in [Appendix 3](#). DSREH recommendations provided in this document will be reviewed in the context of the USEPA recreational criteria/swimming advisories for these cyanotoxins when they are finalized.

Recommended action level and health advisory guidance levels

Action level based on cyanobacterial cell count

Low concentrations of cyanobacteria may cause **allergenic and/or irritative** effects to a portion of an exposed population. These effects are caused by endotoxins (mainly the lipopolysaccharide component of the cyanobacterial cell wall) rather than cyanotoxins. Therefore, county or local authorities may wish to post advisories for any freshwater lake or pond in which cyanobacterial cell counts reach a level of concern.

DSREH recommends that if the cyanobacterial cell count equals or exceeds **20,000 cells/ml in an area where primary recreational contact is likely to occur**, county or local authorities have the option to post advisory signs. When cell counts exceed this level, monitoring for cyanotoxins should be initiated. This recommendation is based on WHO(2003a) guidance described in detail in [Appendix 2](#).

Health advisory guidance levels for individual cyanotoxins

DSREH recommends the following guidance values for recreational exposure to individual cyanotoxins. Their basis, including derivation of Reference Doses and explanation of exposure assumptions, is provided in [Appendix 4](#).

- **Microcystins: 3 µg/L**
- **Cylindrospermopsin: 8 µg/L**
- **Anatoxin-a: 27 µg/L**

Discussion

The recommended water concentrations are intended to be protective of a range of exposures and are probably highly conservative (i.e., protective) for the exposures most likely to occur. The uncertainties in the risk estimates (discussed in [Appendix 4](#)), as well as the inherent uncertainty in the temporal variability of the toxins in any given waterbody, should be considered when providing advice to the public regarding recreation in affected waterbodies. It should be noted that these recommendations do not address the risk to pets, livestock and wild fauna, nor do they address the risk associated with consuming fish from affected waters or the combined risk from swimming and fish consumption.

It should be noted that multiple congeners of microcystin are produced by cyanobacteria, and that the analytical assay used by BFBM measures multiple microcystin congeners as one value, i.e., it does not measure individual congeners. The recommended guidance value for microcystins in general is based on toxicity of microcystin-LR. Microcystin-LR is one of the more prevalent and toxic microcystin isomers and the toxicological database for other microcystin isomers are insufficient for Reference Dose development. Therefore, it is recommended that the guidance value based on microcystin-LR apply to total microcystins.

Citations for this document including the Appendices are found at the end of the document.

APPENDIX 1: METHODS USED BY BFBM FOR CYANOTOXIN ANALYSIS

Cyanotoxins

Test Method: Microcystins/Nodularins (ADDA), ELISA kit. #520011 (Abraxis, Inc.)

Detection Limit: 0.1 µg/L Reacts with all cyclic peptide toxin congeners.

Interferences: ≥ 5% methanol, ≥ 2.5% seawater.

“Positive results requiring regulatory action should be confirmed by an alternative method.”

Cylindrospermopsin

Test Method: Cylindrospermopsin ELISA kit #522011 (Abraxis, Inc.)

Detection Limit: 0.04 µg/L. Reacts with cylindrospermopsin, deoxycylindrospermopsin.

Interferences: ≥ 20% methanol, ≥20% seawater.

“Positive results requiring regulatory action should be confirmed by an alternative method.”

Anatoxin-a

Test Method: Anatoxin-a ELISA kit #520060 (Abraxis, Inc.)

Detection Limit: 0.15 µg/L. Reacts with (+)anatoxin-a, homoanatoxin-a.

Immediate sample preservation required (anatoxin-a will degrade in presence of light, high pH).

Interferences: ≥2.5 % methanol.

“Positive results requiring regulatory action should be confirmed by an alternative method.”

APPENDIX 2: WORLD HEALTH ORGANIZATION (WHO) GUIDANCE FOR RECREATIONAL EXPOSURE TO ALGAL BLOOMS AND CYANOTOXINS

WHO (2003a) has developed guidance based on the concentration of cyanobacteria cells, chlorophyll-a, and one type of microcystin, microcystin-LR. These guidance values form the basis for recreational water advisories adopted by many states. Microcystins are liver toxins. In addition to causing acute liver toxicity, microcystins are promoters for liver carcinogenicity. Additionally, a recent study published subsequent to the WHO (2003a) guidance suggests that microcystins may also cause nephrotoxicity, impairing renal function (Lin. et al., 2016).

WHO (2003a) states that approaches to recreational water safety **should address the occurrence of cyanobacteria as such** (i.e., **cell counts**) for the following reasons: (1) It is unclear whether all important cyanotoxins have been identified. (2) The health outcomes observed after recreational exposure, particularly irritation of the skin and mucous membranes, are probably related to cyanobacterial substances other than the well-known toxins.

Depending upon dose, there are comparatively mild (e.g., irritation) effects in addition to adverse health effects related to toxicity of the cyanotoxins. To address these various effects, WHO (2003) developed three guidance levels.

1. Low probability of adverse health effects

Guidance: A level of **20,000 cyanobacterial cells/ml** (equivalent to **10 µg/L of chlorophyll-a**) for protection for a relatively low probability of **irritative and allergenic effects**. This level would be associated with **2-4 µg/L of microcystin-LR if microcystin-producing cyanobacteria are dominant, with 10 µg/L possible in highly toxic blooms**. At this level, less than 30% of people experienced symptoms, although a small number of people had mild irritation at lower levels (5,000 cells/ml).

Recommended action: Inform authorities to initiate further surveillance of the site. The WHO notes that it is difficult to define “safe” concentrations of cyanobacteria in recreational water for allergenic effects or skin reactions, as individual sensitivities vary greatly.

2. Moderate probability of adverse health effects

Guidance: A level of **100,000 cyanobacterial cells/ml** (equivalent to **50 µg/L of chlorophyll-a**) for potential skin irritations and gastrointestinal effects. This level would be associated with **20 µg/L of microcystin-LR**, assuming an “average” microcystin content per cell, **with 50-100 µg/L possible in highly toxic blooms**.

The 20 µg/L human health value for microcystin-LR is based on the Tolerable Daily Intake (TDI, similar to Reference Dose) of 0.04 µg/kg/day and recreational exposure assumptions for adults (60 kg body weight, ingestion of 100 ml per swimming event). The TDI was developed by WHO, 2003b for its drinking water guidance of 1 µg/L) and is based on liver toxicity in a subchronic (13 week) mouse study (Fawell et al., 1994).

The risk of liver toxicity is primarily from incidental ingestion during swimming, playing in the water, water skiing, boating, etc. Dermal exposure is very unlikely to cause liver toxicity. WHO (2003a) notes that the 20 µg/L is based on adult exposure assumptions and that a 15 kg child could consume 250 ml of water in a swimming event and thus receive 10-times the exposure of an adult from recreational activities. It is also noted that there is increased risk to people with liver disease such as chronic hepatitis B.

Recommended actions: Post warning signs, discourage swimming, daily inspection to watch for scum formation, inform authorities.

3. High probability of adverse health effects

Guidance: Cyanobacteria **scum-containing waters**. Potential for acute poisoning in addition to any of the symptoms noted at the lower levels. Surface scums can represent thousand-fold to million-fold concentrations of cyanobacterial cell populations. The **ingestion of 5–50 mg of microcystin could be expected to cause acute liver injury in a 10-kg child**. Reports of up to 24 mg microcystin/litre from scum material have been published.

Recommended actions: prohibit all water-contact activities, inform authorities.

APPENDIX 3: STATE GUIDANCE/ACTION LEVELS AND RECOMMENDED ACTIONS FOR RECREATIONAL EXPOSURE TO HABs AND CYANOTOXINS

USEPA has summarized recreational water guidance/action levels and recommended actions developed by 21 states for cyanobacteria and cyanotoxins at <http://www2.epa.gov/nutrient-policy-data/policies-and-guidelines#what3>. It should be noted that this state list may not be current in some cases, and that additional states may have developed guidance values since USEPA posted its list. For example, Pennsylvania adopted Ohio's guidance values for Lake Erie.

State guidance/action levels are based on various parameters including visual assessment of the presence of scum, mats, and/or discolored water, cyanobacterial cell counts, and/or concentrations of microcystin-LR and other cyanotoxins. For advisories based on microcystin-LR concentrations, most but not all states use the WHO toxicity factor (tolerable daily intake, equivalent to Reference Dose) of 0.04 µg/kg/day as their basis. However, many states use different exposure assumptions than WHO and have developed values lower than the 20 µg/L WHO value. Specifically, a number of states have guidance values lower than 20 µg/L based on assumptions for recreational exposures for children rather than adults, in order to be public health protective.

Recommended actions may include various levels of recommended/mandatory restrictions based on the level of cyanobacteria or toxins that are present (e.g. posted advisories, recommendations or reducing or avoiding contact with the water, or closure of swimming areas). As with the WHO, some states have tiered guidance levels. For example, Ohio's Public Health **Advisory** Warning recommends no swimming and wading at 6 µg/L for microcystin (with guidance levels also for 3 other toxins) and gives precautions for the very young, the very old, and those with compromised immune systems at these levels. At higher levels there is a more stringent Recreational **No Contact** Advisory.

Listed below are some websites with information on recreational exposure to HABs and cyanotoxins:

California:

http://www.swrcb.ca.gov/water_issues/programs/peer_review/docs/calif_cyanotoxins/cyanotoxins053112.pdf

Indiana: <http://www.in.gov/idem/algae/>

Kansas: http://www.kdheks.gov/algae-illness/download/HAB_response_plan.pdf

Massachusetts: <http://www.mass.gov/eohhs/docs/dph/environmental/exposure/protocol-cyanobacteria.rtf>

Ohio: <http://epa.ohio.gov/Portals/35/hab/beachmanagersguide.pdf>

Oregon:

<http://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Documents/HABPublicHealthAdvisoryGuidelines%2020150619.pdf>

Rhode Island:

<http://www.dem.ri.gov/programs/benviron/water/quality/surfqw/pdfs/cyan1013.pdf>

Vermont: http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf

Virginia:

<http://www.vdh.virginia.gov/epidemiology/DEE/HABS/documents/VDHMicrocystisGuidance.pdf>

Washington: <http://search.usa.gov/search?affiliate=dohwagov&query=cyanotoxins>

The basis for guidance values developed by states, USEPA, and WHO for the three cyanotoxins evaluated herein is presented in the tables below:

Microcystins

Agency	Year	Form	RfD (µg/kg/day)	Exposure Assumptions			Guidance value (µg/L)
				Body weight (kg)	Water ingestion rate (L/hr)	Duration of swimming event (hrs)	
California EPA	2012	-LR, LA, - RR, -YR	0.006	30.25	0.05	5	0.8
New Jersey DEP	2017	Total	0.01	31.8	0.12	1	3
Ohio DOH	2015	Total	0.04	15	0.05	2	6
Oregon Health Authority	2015	Not stated	0.05	20	0.05	2	10
USEPA*	2016	Total	0.05	31.8	0.33	2.7	4
WHO**	2003	Total	0.04	60	0.1 (per swimming event)		20

*From USEPA Draft Human Health Recreational Ambient Water Quality Criteria (USEPA, 2016).

**Adult exposure assumptions; noted that 15 kg child could receive 10-fold higher exposure

Cylindrospermopsin

Agency	Year	RfD (µg/kg/day)	Exposure Assumptions			Guidance value (µg/L)
			Body weight (kg)	Water ingestion rate (L/hr)	Duration of swimming event (hrs)	
California EPA	2012	0.033	30.25	0.05	5	4
New Jersey DEP	2017	0.03	31.8	0.12	1	8
Ohio DOH	2015	0.03	15	0.05	2	5
Oregon Health Authority	2015	0.1	20	0.05	2	20
USEPA*	2016	0.1	31.8	0.33	2.7	8

*From USEPA Draft Human Health Recreational Ambient Water Quality Criteria (USEPA, 2016)

Anatoxin-a

Agency	Year	RfD (µg/kg/day)	Exposure Assumptions			Guidance value (µg/L)
			Body weight (kg)	Water ingestion rate (L/hr)	Duration of swimming event (hrs)	
California EPA*	2012	2.5	30.25	0.05	5	90
New Jersey DEP	2017	0.1	31.8	0.12	1	27
Ohio DOH	2015	0.5	15	0.05	2	80
Oregon Health Authority	2015	0.1	20	0.05	2	20

*Considered dermal and inhalation exposure in addition to ingestion exposure. Dermal exposure was about twice ingestion exposure. Inhalation exposure was not significant.

APPENDIX 4

DERIVATION OF HEALTH ADVISORY GUIDANCE LEVELS FOR CYANOTOXINS

March 10, 2017

Derivation of Reference Doses (RfDs)

The studies summarized here appear to be the only relevant mammalian *in vivo* oral dosing studies available for each of the hazardous algal toxins addressed in this assessment. They include all of the oral exposure studies addressed by WHO and the other states referenced in appendix 3 and other relevant studies cited in the USEPA Health Advisories and Support documents for microcystins, cylindrospermopsin and anatoxin-a (USEPA, 2015a-e).

The assessment of these cyanotoxins is limited to those animal studies that administered the toxin as the specifically isolated chemical. It is also generally limited to those studies in which the toxin was administered orally, either by gavage or through drinking water. Data from studies that included injection sub-studies are included where those data are useful in informing the results of the oral administration. As explained in the Exposure Scenario section, only studies with less than sub-chronic to sub-chronic duration were considered for quantitative derivation of an RfD. Finally, these assessments are limited to studies that evaluated at least one endpoint suitable for RfD development. Studies that only presented data on sub-clinical or mechanistic endpoints are not considered here.

I. Microcystin-LR (MC-LR)

Review of toxicologic data:

*Fawell et al., (1999)*² - Mice (CD-1) were exposed to MC-LR obtained from a commercial laboratory. (This study also initially investigated rats as well as mice, but mice were found to be a more sensitive species). It was stated that MC-LR was obtained from a commercial laboratory as a “pure” substance, but no further details were provided. Fawell et al. (1994) do not indicate that the material was tested for purity. As MC-LR is a (hepta)peptide, this is somewhat less of a concern than for more structurally complex chemicals. However, this does introduce some uncertainty into the quantitative assessment of the reported results.

Single dose study: Groups of 5 male and female mice were given a single dose of MC-LR in aqueous solution by gavage (500, 1580, 5000 µg/kg) or by intraperitoneal injection (50, 158, 500 µg/kg). This study was intended as a range-finding study and no control groups were used. By

² The Fawell et al. (1999) paper provides a journal-based version of the report-based Fawell et al. (1994) report-based version of this research. There does not appear to be significant disagreement between these two publications, but the report-based version is more complete.

oral exposure, there was no mortality among males at the two lower doses and 60% mortality at the high dose. Among the females, there was no mortality at the low dose, and 20% and 40% at the two highest doses. By intraperitoneal (i.p.) injection there was no mortality at the lowest dose and 100% mortality for males and females at the two highest doses. The oral LD₅₀, therefore, is approximately 5,000 µg/kg.

Liver effects from the single dose study were also examined. For the oral dose, “minimal” diffuse hemorrhage was observed at the low (500 µg/kg) dose in 2/5 males, and in 1/5 at each of the two highest doses. There were also 2/5 and 5/5 observations of “moderate” centrilobular hemorrhage in 2/5 males at 1580 µg/kg and 5/5 males at 5000 µg/kg, and in 2/5 females at 5000 µg/kg. In addition, centrilobular necrosis was observed in 1/5 males and females at the highest dose. Diffuse hemorrhage was also evident at all i.p. doses.

It is clear that the liver is a target for MC-LR. The apparent LOAEL for acute liver effects is 500 µg/kg from oral exposure for minimal diffuse hemorrhage in males, with no NOAEL identified; although the lack of this effect at higher doses and with repeated dosing makes this conclusion somewhat unclear. It should be noted that diffuse hemorrhage occurred at lower doses (50 and 158 µg/kg) from i.p. injection.

Developmental study: Pregnant female mice (n = 14-26/dose group) were exposed by gavage from GD 6-15 to 0, 200, 600, or 2,000 µg/kg/d in aqueous solution with necropsy on GD 18. In the high dose group, 9 females died or were sacrificed *in extremis*. Body weight gain among surviving dams was not affected. No effects are reported for the dams exposed at the two lower doses. The number of implantations, live fetuses, and post-implantation loss were not clearly affected by MC-LR. There was a small (6%), but significant reduction, in fetal weight in males and females compared to controls at the highest maternal dose, but no clear effect on fetal weight at the other doses. There was no clear effect of MC-LR on fetal visceral or skeletal structure at any dose.

MC-LR does not appear to be strongly fetotoxic. The high maternal dose (2,000 µg/kg/d) appears to have a mild effect on fetal weight gain and should be considered a LOAEL for fetal effects, although this dose also resulted in maternal lethality. The NOAEL is, therefore, 600 µg/kg/d.

Less than sub-chronic duration study (14 days): Male and female mice (5/sex/dose group) were exposed by gavage daily to 0, 40, 200, or 1,000 µg/kg/d (presumably in aqueous solution, as this was the method in the longer-term follow-up 13 week study) for 14 days. Histopathology was conducted on lung and liver. Organ weights were not determined. There was no dose-related mortality, and there were no effects on body weight, body weight gain, or food consumption. Macroscopic findings were not remarkable with the possible exception of red discoloration of an accentuated lobular pattern on the liver for 2/5 males at 1,000 µg/kg/d, and a red discoloration of

the lungs for 1/5 males at 40 µg/kg/d and 2/5 males at 1,000 µg/kg/d. Histopathology found hepatic centrilobular cellular rarefaction (decreased density of cell count) at 1,000 µg/kg/d.

1,000 µg/kg/day appears to be a minimal LOAEL and 200 µg/kg/d the NOAEL from this study.

Sub-chronic study (13 weeks): Males and female mice (15/sex/dose group) were exposed by daily gavage to 0, 40, 200, or 1,000 µg/kg/d in aqueous solution for 13 weeks (91 d). Animals were assessed for body weight; food consumption; hematology; blood chemistry; organ weights (adrenals, kidneys, liver and testes); organ/tissue pathology; and histopathology. For males at 1,000 µg/kg/d, there was one death on day 7 with no obvious pathology and one case of frank neurologic morbidity at day 91. There was a significant decrease in body weight gain (15%) at the 40 and 200 µg/kg/d doses, and body weight was also decreased at the 1,000 µg/kg/d dose but was not statistically significant. For females, there was a significant (29%) *increase* in body weight at the 200 µg/kg/d dose only. For both males and females, there was a significant increase in food consumption (14% and 20%) at 1,000 µg/kg/d.

In females, there was a significant increase (10-12%) in hemoglobin concentration, RBC count, and packed RBC volume at 1,000 µg/kg/d. In males, liver enzyme concentrations in blood were significantly increased at 200 µg/kg/d (ALT, AST) and 1,000 µg/kg/d (ALP, ALT, AST). There was also an overall decrease in gamma-glutamyl transferase (GGT), but this was significant only for males at 200 µg/kg/d. Total blood protein and albumin were also significantly decreased at 200 and 1,000 µg/kg/d in males. Although not significant, there was also a decrease in these two parameters at 40 µg/kg/d. No dose-related effects on organ weight were observed. Significant histopathological effects were seen only in the liver. For both males and females, there was a monotonic increase in the incidence of generalized chronic liver inflammation. The authors did not provide an analysis of statistical significance for this observation. However, a chi-squared analysis indicates that for males and females, there was a statistically significant difference only between the controls and the 1,000 µg/kg/d dose.

Although 200 µg/kg/d is a clear effect level in this study based on decreased weight gain in males, increased serum liver enzymes, and decreased total blood protein, and albumin in males, it is not entirely clear from these data that 40 µg/kg/d is a NOAEL. This conclusion is based on the significant decrease in body weight gain in males, and is also supported by changes in other parameters (total blood protein, albumin, and chronic liver inflammation) that were not statistically significant at this dose. Therefore, it appears appropriate to characterize 40 µg/kg/d as a minimal LOAEL.

Heinze (1999) - Adult male hybrid rats (WELS x BDIX) received MC-LR [Calbiochem] through drinking water at doses of 50 or 150 µg/kg/d. There were 10 animals/dose group including controls. Animals were sacrificed after 28 days of exposure. Measurements consisted of: body weights; organ weights (liver, kidneys, adrenals, thymus, and spleen); erythrocyte and leukocyte

counts; hemoglobin concentration; hematocrit; and serum enzymes. Histopathological examinations were conducted on liver and kidney.

There was no dose-related effect on body weight. Relative liver weight was significantly increased relative to controls at both doses. There were no other significant changes in organ weight. There was a significant increase in leukocytes and lymphocytes at the 150 µg/kg/d dose only. There was a significant increase in the serum levels of LDH and ALP (but not ALA or AST) at both doses, suggesting liver toxicity. Liver pathology, characterized by the author as “toxic hepatitis” was observed diffusely throughout the parenchyma at both doses. This included degenerative and necrotic hepatocytes (with and without hemorrhages).

This study yields clear evidence of liver damage at both doses and evidence of hematologic abnormalities at the high dose. Based on these observations, 50 µg/kg/d is a clear LOAEL. No NOAEL was identified from this study. It should be noted that this study was chosen by the USEPA (2015a) as the basis for its Health Advisory for MC-LR.

Li et al. (2014) – Adult male Sprague-Dawley rats were exposed by gavage to commercial MC-LR (reported as > 95% purity) mixed with methanol and diluted to administered concentrations with distilled water. The reported administered amounts were 0, 0.2, 1.0, or 5.0 µg/kg every two days for a total of 8 weeks (56 d), corresponding to effective doses of 0, 0.1, 0.5, and 2.5 µg/kg/d (n = 8/dose group). The primary analysis in this study was the potential effect of MC-LR on spatial learning as measured in the Morris water maze test administered 24 hrs after the final dosing. In addition, 24 hrs after the behavioral studies, blood was drawn and analyzed for serum liver enzymes, cholinesterase, total protein and albumin. The rats were sacrificed 24 hrs after the completion of the behavioral studies. Histopathology was conducted on the neurons of the hippocampal region of the brain, and on the liver.

Rats showed decreasing performance on most of the Morris water maze parameters as a function of dose, with significant decrements in the 2.5 µg/kg/d group and a significant decrement in the 0.5 µg/kg/d group in one parameter (platform zone frequency). Serum cholinesterase was significantly increased at 2.5 µg/kg/d. No significant treatment related histopathology was noted in either the hippocampus or the liver. However, the authors did report an increase in the density of N-20+ cells in the hippocampus as a function of dose. This effect reached statistical significance relative to the control animals only at the highest dose, however. N-20+ is an immunologic marker for the expression of the NOS2 gene that codes for nitric oxide synthetase. This gene is inducible by cytokines and the increase of N-20+ cells was interpreted by the authors as an indication of an inflammatory response in the hippocampus. The interpretation of the toxicological significance of this observation, however, is not entirely clear.

Based on the performance decrements in the Morris water maze test, 0.5 µg/kg/d appears to be a LOAEL for neurologic effects from this study, and 0.1 µg/kg/d is the NOAEL. However, as the USEPA (2015c) points out, the administered doses of MC-LR also delivered doses of methanol

that increased in proportion to the administered MC-LR dose, but given the lack of data on the volume of the gavage solution, the actual methanol dose cannot be determined. Methanol is a known neurotoxicant and the potential for a synergistic effect of methanol and MC-LR cannot be ruled out.

Li et al. (2015) – Adult female Sprague-Dawley rats (n = 7/dose group) were dosed by gavage with MC-LR (mixed with methanol and diluted with distilled water) at 0, 1.0, 5.0 or 20.0 µg/kg every two days for 8 wks. This resulted in effective doses of 0, 0.5, 2.5, or 10.0 µg/kg/d. One day following the final dosing, the rats were mated with unexposed males. Maternal body weight was recorded during gestation, and reproductive parameters were recorded. From each litter, 4 M and 4 F pups at PND 7 were evaluated in a series of behavioral tests: motor development (surface righting, negative geotaxis, cliff avoidance); and at PND 28 and 60 were evaluated for open field and Morris water maze. Histopathology was also conducted on one male and one female pup brain from each dam 24 hrs after each behavioral test.

There was no treatment related maternal toxicity and no effect on maternal body weight. The number of pregnant females appears to have declined and the number of dead fetuses appears to have increased with MC-LR dose. However, these parameters did not differ significantly from controls. Performance of the pups in the cliff avoidance test declined significantly at all doses. Pup performance in the Morris water maze was negatively affected by treatment. This was most evident with respect to the frequency-in-platform-zone parameter which showed statistically significant reduced performance at all doses. No treatment related effect was seen in brain histopathology. There was, however, an increase in markers of oxidative stress in the hippocampus that were significant at the highest dose and to a lesser extent at 2.5 µg/kg/d.

The apparent LOAEL for neurological developmental effects in this study was 0.5 µg/kg/d. There was no NOAEL. However, as with the related Lin et al. (2014) study, MC-LR exposure was associated with methanol exposure, although the actual methanol dose cannot be determined. It is, therefore, difficult to determine the specific effect of MC-LR alone. It should also be noted that the exposure of the females in this study was only pre-conception. While this does not, by itself, limit the applicability of these data for the purpose of identifying an adverse effect, it does make interpretation of the nature of the effects of MC-LR difficult. Because the pharmacokinetics of MC-LR in these animals is unknown, the fetal doses cannot be estimated.

Zhang et al. (2010) – Adult male C57bl/6 mice received commercial MC-LR in drinking water. The purity of the MC-LR was stated to be > 95%. The mice were exposed for 180 days, making this a chronic exposure study. The drinking water concentrations were 0, 1, 40 and 80 µg/L which, according to the authors, corresponded to doses of 0, 0.2, 8.0 and 16.0 µg/kg/d with n = 10 for each group. The primary purpose of this study was to investigate the effect of MC-LR exposure on the expression of matrix metalloproteinases. These are a family of enzymes that function in degrading the extracellular protein matrix. As such, they are linked to tumor progression by providing space for tumor expansion. However, because this assessment focuses

on short-term exposure, tumor progression is not considered a relevant endpoint and changes in matrix metalloproteinase expression are, therefore, considered to be a mechanistic or sub-clinical endpoint that is not appropriate for short-term RfD development. Nonetheless, this study also addresses outcome determinations relevant to RfD derivation: body weight, liver weight, and liver histopathology.

The authors report a statistically significant decrease in body weight and an increase in relative liver weight compared to the controls in the 8.0 and 16.0 $\mu\text{g/kg/d}$ dose groups. However, the specific data are not presented. Histopathological examination of the livers revealed infiltration of lymphocytes and fatty degeneration in the 8.0 and 16.0 $\mu\text{g/kg/d}$ dose groups.

While this study confirms the hepatotoxic potential of MC-LR, as a chronic duration study, it is not quantitatively applicable for derivation of a shorter duration RfD.

Ueno et al. (1999) - Adult female BALB/c mice were exposed to MC-LR isolated from “algal bloom materials and stated to have > 95% purity. Exposure occurred through drinking water for 3 (n = 20), 6 (n = 20), 12 (n = 20) or 18 months (n = 40) at a single concentration of 20 $\mu\text{g/L}$ plus controls. The mean cumulative MC-LR intake over 18 months (548 days) was reported as 35.5 $\mu\text{g/animal}$. The mean body weight for the animals is not reported. However, the graphical presentation of body weight over the duration of exposure yields an estimated time-weighted body weight of 25.3 g (0.025 kg). Thus the dose of MC-LR can be estimated as 2.6 $\mu\text{g/kg/d}$. This study is considered a chronic duration study for all exposures except for the 3 month exposure. Analyses included body weight; organ weights; hematology; serum chemistry - liver enzymes, serum glucose, lipids, bilirubin, Ca, and inorganic P. Histopathology was performed on a large number of tissues.

There was no significant difference from controls in survival, body weight, or food or water consumption at intermediate time points or at the termination of the exposure. No significant differences were noted in hematology throughout the study. The only statistically significant treatment related effects seen in serum chemistry were a transient decrease in ALP at 12 months and an increase in total cholesterol at 18 months. A significant decrease in relative thymus weight was observed at months 3-12, but not at 18 months. A significant decrease in absolute (but not relative) heart weight was observed 18 months, but not at earlier time periods. No remarkable outcomes were observed in histopathological analysis (including histopathology of the liver)

Overall this study did not show significant adverse outcomes from MC-LR, and a dose of approximately 2.6 $\mu\text{g/kg/d}$ can be considered to be a chronic NOAEL. However, given the chronic nature of this exposure, this cannot reliably be back-extrapolated to a shorter term RfD. It can, however, be useful in informing an RfD derived from a less than chronic study, especially for liver histopathology, as a NOAEL from a chronic duration study can be expected to be a lower bound estimate for a NOAEL from a less-than-chronic duration study.

Chen et al. (2011) – Adult male SPF mice (age not specified) were orally administered commercial MC-LR daily for either 3 or 6 months. The purity of the material was not provided by the authors. The route of exposure appears to be through drinking water, but this is not explicitly stated. The daily administered concentrations were 0, 1, 3.2, or 10 µg/L (n = 20/dose group). Although a range of body weights is given, the body weights over time are not specified. Thus, the dose (µg/kg/d) cannot be directly calculated from the published data. The USEPA (2015c), in its review of this study, estimates the corresponding daily doses on the basis of species/strain-specific default assumption about water intake and body weight as 0, 0.25, 0.79, and 2.5 µg/kg/d. The 6-month duration exposure is considered to be a chronic duration study. Body weight and testis weight were measured. Sperm count and sperm morphology were assessed, and testis histopathology was evaluated. Serum reproductive hormones were also measured.

No treatment-related effects were observed on body weight or testis weight. At 3 months, a significant decrease in sperm motility was observed at 0.79 and 2.5 µg/kg/d. At 6 months, there was a decrease in sperm count and sperm motility as well as an increase in the frequency of abnormal sperm for 0.79 and 2.5 µg/kg/d. Although serum testosterone appears to have markedly declined for 2.5 µg/kg/d at three months, it apparently did not reach a level of statistical significance. At 6 months, serum testosterone was statistically significantly decreased at 0.79 and 2.5 µg/kg/d. Also, LH was significantly increased at the same doses, while serum FSH was significantly increased at 2.5 µg/kg/d. Histopathology revealed a slight effect on the arrangement of spermatogenic epithelium in the seminiferous tubules at 2.5 µg/kg/d at 3 months. At 6 months, there was slight testicular atrophy at 0.79 µg/kg/d with increasing severity and various morphological abnormalities at 2.5 µg/kg/d.

This study suggests the potential for male reproductive toxicity from chronic MC-LR exposure with an apparent NOAEL of 0.25 µg/kg/d. However, a number of factors render this study problematic for RfD development. As noted above, the dose (µg/kg/d) could not be determined directly from the published data. The age of the mice was not provided and sperm quality can vary as a function of age. There are several potentially significant methodological issues with sperm and tissue analysis, as pointed out by the USEPA (2015c).

Lin et al. (2016) - This was a cross sectional epidemiology study in a population in Southwest China exposed to both MC-LR and aflatoxin. Renal function indicators (blood urea nitrogen, BUN; serum creatine, SCr; estimated glomerular filtration rate, eGFR) were evaluated as a function of estimated MC-LR (and aflatoxin) intake from water and food. The population in this study was apparently exposed long-term (possibly over a lifetime) to microcystin-LR that appears to have been chronically (or at least repeatedly) present in its environment. The mean, median, 75th and 95th percentiles of estimated daily MC-LR intake were 4.05, 3.23, 4.66, and 9.55 ng/kg/d, respectively.

For both the full study population (5,493 people) and the subset of those with abnormal renal function (129-383 people depending on the specific renal function parameter), there was a significant association of renal function indicators with estimated MC-LR (but not aflatoxin), with an apparent dose-response relationship across quartiles (significant for trend) of MC-LR exposure. This association was seen for each renal function indicator in adjusted models. The odds ratio (OR) for having abnormal renal function indicators was significantly > 1.0 for the third and fourth quartiles of estimated MC-LR intake. The OR for having abnormal renal function indicators relative to the median estimated MC-LR intake in the fully adjusted model was significantly > 1.0 for all three renal function indicators. Although there is documented exposure to aflatoxin in this population, this did not appear to confound the observed associations with MC-LR.

Although this study does not readily lend itself to the calculation of a NOAEL, based on the above summary, the lowest quartile of exposure (i.e., $0.003 \mu\text{g/kg/d}$) appears to be the most reasonable estimate of the estimated exposure that is not clearly associated with adverse effects. Nonetheless, the reliability of the exposure estimates in this study is not clear and there was no independent estimate of intake that could be used to ground-truth these estimates. Thus, the quantitative determination of dose-response from this study can be viewed as only suggestive. Importantly, this study suggests that MC-LR is associated with adverse renal function in humans.

RfD Derivation:

Selection of critical study for derivation of an RfD – General considerations

The two Li et al. studies (2014, 2015) on rats both suggest the potential for MC-LR to cause both adult and developmental neurotoxicity with sub-chronic exposure. The apparent LOAEL for both endpoints is $0.5 \mu\text{g/kg/d}$. However, interpretation of the results of both studies is complicated by co-exposure to methanol and the possibility that the co-exposure could result in a synergistic response.

The Chen et al. (2011) study suggests the potential for male reproductive effects with an apparent NOAEL $0.25 \mu\text{g/kg/d}$. However, as discussed above, there are numerous reporting and methodological issues with this study.

The Lin et al. (2016) study has the advantage of investigating a human population. However, the accuracy of the microcystin-LR exposure estimates in that study is unknown. Furthermore, the statistical analysis does not readily lend itself to the estimate of a NOAEL. In addition, although not entirely clear from the published paper, it appears that this population was chronically exposed to microcystin-LR. Thus, the estimated dose-response relationship from this study may not be appropriate for the purposes of deriving a short-term RfD. Both the Fawell et al. (1994/1994) and Heinze (1999) studies provide appropriate toxicological data for the derivation of an RfD. These studies yield a LOAEL at very similar doses, with no NOAEL identified.

In the Fawell et al. (1994/1999) study, the 40 µg/kg/d dose is identified as a minimal LOAEL based on the significant decrease in body weight gain in males, and is also supported by changes in other parameters (total blood protein, albumin, chronic liver inflammation) that were not statistically significant at this dose, but are predictive of significant effects at higher doses. This study also provides information on effects from developmental exposures. In the Heinze (1999) study, the 50 µg/kg/d dose is a clear LOAEL that reflects liver toxicity based on increased liver weight and elevated serum liver enzymes. Liver toxicity was also observed in the Fawell et al. (1994/1999) study, but only appears to have reached statistical significance at 1,000 µg/kg/d. Although the LOAEL from the Fawell et al. (1994/1999) study (40 µg/kg/d) is slightly lower than the LOAEL from the Heinze (1999) study (50 µg/kg/d), the LOAEL from Fawell et al. (1994) is judged to be a minimal LOAEL, whereas the LOAEL from Heinze (1999) is a LOAEL for more significant adverse effects. In addition, the length of exposure in the Heinze (1999) study (28 days) was less than that in the Fawell et al. (1994/1999) study (91 days). However, the USEPA (2015a) cites a study (Guzman and Solter, 1999) in which rats were intraperitoneally infused with MC-LR. In that study, the route of exposure resulted in direct exposure of the liver. Adverse liver effects were observed in that study, and the NOAEL and LOAEL doses were separated by a factor of two. The USEPA thus argues that a full factor of 10 is not necessary to estimate the NOAEL from the observed LOAEL for adverse effects in the Heinze (1999) study, and the alternative UF of 3 is appropriate. Given these considerations it appears more appropriate to identify the minimal, but lower LOAEL of 40 µg/kg/d for small, but significant decreased body weight in male mice in Fawell et al. (1994/1999) as the point of departure for RfD derivation, noting that applying a UF of 3 to estimate the NOAEL from the minimal LOAEL in Fawell et al. (1994/1999) also adequately addresses the NOAEL for liver effects in the Heinze (1999) study based on the argument presented by the USEPA (2015a).

Uncertainty factor analysis - A total UF of 3,000 was applied to the LOAEL based on the following individual UFs:

UF – study duration = 1

Although this was a sub-chronic duration study, it appears appropriate to the relevant exposure scenarios.

UF – LOAEL-NOAEL = 3

The moderate decrease in male body weight gain at the 40 µg/kg/d dose is identified as a minimal LOAEL.

UF – animal-human = 10

Standard assumption - this includes factors of 3 each for interspecies toxicokinetic and toxicodynamic variability.

UF – sensitive human populations = 10

Standard assumption – includes children as a sensitive group.

UF – database = 10

The only studies that address neurotoxicity/developmental neurotoxicity are the two studies of Li et al. (2014, 2015). Both of these studies yield a LOAEL of 0.5 µg/kg/day. The interpretation of both of these studies is potentially confounded by co-exposure to methanol, a known neurotoxicant. However, the extent of confounding by methanol in either Li et al. study is unknown, and the neurotoxicity and developmental neurotoxicity effects in these studies could, in fact, be independent of the methanol exposure. If it is assumed that the application of a UF of 3 to account for the use of a minimal LOAEL in the absence of a NOAEL from the Fawell et al. (1994/1999) study appropriately estimates a NOAEL from that study (i.e., 13 µg/kg/d), then application of an additional UF of 3 for database uncertainty would yield a value of 4 µg/kg/day (leaving aside the other UFs that are independent of the treatment of the Li et al. studies). This would still be an order of magnitude larger than the LOAEL from the Li et al. (2014, 2015) studies. Alternatively, application of a full uncertainty factor of 10 (rather than 3) to the estimated NOAEL (13 µg/kg/d) from Fawell et al. (1994/1999) to account for database uncertainty regarding the potential for neurotoxicity/developmental toxicity would result in a value of 1.3 µg/kg/d. This value is still approximately twice the LOAEL from the Li et al. studies. The application of a full UF of 10 for database uncertainty also appears to address all other database issues.

UF-total = 3,000

RfD calculation

$$\begin{aligned}\text{RfD} &= \text{LOAEL} \div \text{UF-total} \\ &= 40 \text{ µg/kg/d} \div 3,000 \\ &= 0.013 \text{ rounded to } \mathbf{0.01 \text{ µg/kg/d}}\end{aligned}$$

Comparison to USEPA RfD and WHO Tolerable Daily Intake (TDI) - The RfD of 0.01 µg/kg/d is smaller than the Tolerable Daily Intake (TDI) (0.04 µg/kg/d) developed by WHO (2003b) as well as the RfD value developed by the USEPA (2015a) for MC-LR of 0.05 µg/kg/d. The difference is almost entirely due to the consideration of the database uncertainty regarding the potential for neurotoxicity/developmental neurotoxicity introduced by the Li et al (2014, 2015) studies. This uncertainty was not addressed directly by either WHO or USEPA.

II. *Cylindrospermopsin (CYN)*

Review of toxicological data:

Humpage and Falconer (2003) - Male mice (Swiss albino) were dosed with cylindrospermopsin derived from *C. raciborskii* cells (strain AWT 205) in overlapping studies, the first with

exposure by drinking water for 10 weeks with a CYN dose range of 216-657 µg/kg/d, and the second by gavage for 11 weeks with a dose range of 30-240 µg/kg/d. The drinking water study was conducted using a crude cell extract, while the gavage study utilized a purified extract, assayed as 47% CYN, 53% phenylalanine. The n for controls was 12 in the drinking water study and 10 for the gavage study. The n was 10 for all dose groups except the highest doses, which had an n of 5-6. The study protocols appear valid although the body weights of the controls at sacrifice in the two studies differed despite being in the same range at the start of dosing. There was a 23% decrease in body weight of gavage controls compared to the drinking water controls. The authors speculate that this was due to discomfort resulting from the gavage treatment *per se*.

In the drinking water study, body weight was significantly decreased at the two highest doses, and liver and kidney weight were significantly increased at all doses. Spleen weight was not significantly affected. At the two lowest doses, 30 and 60 µg/kg/d, in the gavage study, body weight was significantly increased compared to controls, but it was significantly decreased at the two highest doses in the drinking water study. Relative liver weight increased monotonically with an apparent LOAEL of 216 µg/kg/d in the drinking water study, and a NOAEL and LOAEL of 120 µg/kg/d and 240 µg/kg/d, respectively, in the gavage study. In the drinking water study, kidney weight increased monotonically and significantly across all dose groups compared to controls. In the gavage study, kidney weight increased monotonically, with a LOAEL of 60 µg/kg/d and a NOAEL of 30 µg/kg/d (the lowest dose). There were no other significant changes in organ weight. Urine protein/creatinine ratio decreased monotonically with a LOAEL of 120 µg/kg/d in the gavage study. There was minimal liver histopathology (unspecified) at 120 and 240 µg/kg/d, but no changes in serum liver enzymes in the gavage study. Serum bilirubin was significantly increased and serum total bile acids were significantly decreased at 216 and 432 µg/kg/d in the drinking water study, but were not significantly altered in the gavage study.

The authors hypothesize that CYN inhibits protein synthesis. Decreased urinary protein (presumably small proteins, not reflective of glomerular or tubular damage) is consistent with this hypothesis, although the decreased protein/creatinine ratio in conjunction with decreased creatinine concentration at the high dose in the gavage study could reflect increased creatinine due to protein catabolism as well as, or instead of, decreased overall protein synthesis at the high dose.

The study NOAEL is 30 µg/kg/d based on increased relative kidney weight. While increased relative kidney weight is considered an adverse effect, the mode of action leading to the effect in this study is unclear. The authors hypothesize that increased kidney weight could reflect an increase in cellular volume or cellularity in response to inhibition of protein and general metabolic synthesis. While this explanation appears to be speculative, the linkage of this effect to more frank adverse effects at higher doses including decreased body weight argues for the

validity of this NOAEL. However, there would be more confidence in this value if there had been a lower dose that also showed no adverse effect.

Chernoff et al. (2011) – Pregnant CD-1 mice were exposed to commercial CYN (>98% pure) by intraperitoneal injection daily for 5 days at doses of 50 µg/kg/d during GD 8-12 or GD 13-17. Animals were sacrificed (n = 2-5) 24 hrs after the final injection, or on post-treatment days 7 and 14. Measurements included maternal weight, serum chemistry, and histopathology of liver and kidney.

Decreased weight gain in the GD 8-12 group occurred starting with the first dose, followed by vaginal bleeding, reduced activity, blood accumulation in the tail, and hemorrhaging around the eyes. There was mortality and morbidity during dosing with some mortality through GD 18. Gestational length was not affected. These effects were milder in the GD 13-17 group. Treated animals in this group gave birth earlier in the day compared to controls. Serum liver enzymes (AAT, AST, and ALT) were significantly elevated in the GD 8-12 group and in the GD 13-17 group. LDH and SDH were elevated in the GD 13-17 group. Serum albumin was significantly reduced in both groups. BUN and creatinine were significantly increased in the GD 8-12 group, and serum glucose was significantly decreased in both groups. Blood chemistry parameters returned to normal values 7 days post-dosing in both groups. Relative liver weight was not affected in the GD 8-12 group, but was elevated in the GD 13-17 group and did not recover. Minimal-moderate centrilobular hepatocyte necrosis and apoptosis was elevated in both treatment groups compared to controls. Elevated minimal-moderate chronic interstitial nephritis was seen in the GD 8-12 group.

The injection route of exposure and the single dose nature of this study preclude its use in RfD derivation. However, this study provides evidence of the potential of CYN to result in adverse metabolic, liver and kidney effects.

Chernoff et al. (2014) - Pregnant CD-1 mice were exposed to CYN by i.p. injection during either gestation day (GD) 8-12, or GD 13-17. Animals were dosed on five successive days with 50 µg/kg/d of CYN. Groups of dams were sacrificed after each day of dosing and at various times up to 13 weeks post-dosing. Maternal body weight, visual signs of toxicity, serum chemistry, and liver and kidney histopathology were analyzed. The number of animals examined (generally 4-21) varied by endpoint and number of doses.

Maternal weight decreased significantly during the dosing period in both gestational period groups. Vaginal hemorrhaging and visual signs of morbidity were also observed in both groups, although the late gestational period group showed effects after the first dose compared to the third dose in the early gestational group. ALT, SDH, and total bile acids were significantly increased in both groups both during, and to varying extents, post-dosing, indicating liver toxicity. Hemoglobin and hematocrit were reduced in both groups with a greater sensitivity in the later gestational group. Significant increases in liver weight were seen in the late gestational

group. Liver histopathology including hepatocellular necrosis; hepatocellular cytoplasmic alterations; and chronic centrilobular inflammation was observed in both groups. In the kidney, acute tubular necrosis was also observed in both groups. In addition, a significant decreased platelet count was observed during the last two days of dosing.

The injection route of exposure and the single dose nature of this study preclude its use in RfD derivation. However, this study provides further evidence of the potential of CYN to result in metabolic, liver, kidney and hematologic adverse effects.

de Almeida et al. (2013) – Pregnant Wistar rats were exposed to CYN by gavage at 0, 0.03, 0.3 and 3.0 µg/kg/d for GD 1-20 (n = 10/dose group). Body weight was measured during and at the end of treatment. Organ weights were measured for ovaries, uterus, kidney, pancreas, adrenal gland, heart and spleen. Histopathology was conducted on liver and kidney. Reproductive parameters were recorded. Half of the fetuses from each litter were examined for visceral malformation, and the other half were examined for skeletal malformation.

No significant differences from controls were observed for body weight or histopathology. No differences were observed in organ weights, or in the incidence of visceral or skeletal malformations.

This study yields a free-standing NOAEL of 3.0 µg/kg/d for maternal, reproductive and teratological effects from gestational exposure.

Sukenik et al. (2006) – Four-week old ICR mice (M and F) were exposed to a cell-free, but unpurified solution of CYN in water. The exposure protocol in this study is somewhat unusual. In order to minimize the number of animals, the authors gradually increased the dose to all of the animals over the course of the study. Blood was drawn from the tails every four days for determination of hematocrit and cholesterol and half of the animals were sacrificed at 20 weeks and the remainder at 42 weeks. The authors state that the dose increased from 10 µg/kg/d at the start of the study to > 50 µg/kg/d in the last 22 weeks of exposure. Although not explicitly stated in the paper, the dose at the 20-week sacrifice was approximately 30 µg/kg/d based on the graphic presentation in the paper. This study is, therefore, a chronic duration study. The authors state that analysis of the drinking water revealed only CYN and the related compound 7-epi/CYN. However, the purity of the CYN is not known. Blood samples were obtained every four weeks. There were initially 20 males and 20 females in the control and exposure groups. Liver, spleen, kidney and testes were weighed and examined by histopathology. Cholesterol was determined in RBC membranes, plasma, and liver homogenate.

No effect on body weight gain was observed. Relative liver weight was significantly increased at 42 weeks (but not at 20 weeks) in M and F. Relative kidney weight was significantly increased in M and F at both 20 and 40 weeks, and relative testes weight was significantly increased at 42 weeks. Relative spleen weight was not affected. Hematocrit was significantly increased compared to controls in M at all time periods of measurement except for the final (36

week) period when it was significantly decreased compared to controls. Female hematocrit was also significantly increased for all time periods except for the final measurement period (36 weeks). This was accompanied by deformed RBCs. RBC membrane cholesterol was significantly increased in M and F at 42 weeks. Plasma cholesterol was slightly (but significantly) increased in F at 42 weeks. In liver homogenate, however, cholesterol was significantly decreased in M at 20 weeks and in M and F at 42 weeks.

This study suggests that chronic exposure to moderate levels of CYN can result in adverse effects in liver, kidney and testes weight, hematologic parameters and cholesterol levels. However, the chronic nature of this study renders it not appropriate for derivation of a shorter term RfD. Additionally, the inability to link effects to specific CYN doses and the potential contribution of the 7-epi/CYN compound precludes the use of this study for quantitative assessment for CYN.

Rogers et al. (2007) - Pregnant CD-1 mice were injected (i.p.) with 8-128 µg/kg/d CYN, stated to be free from organic impurities and with >98% purity, during GD 8-12. Term fetuses were examined for viability and structural abnormalities. Significant lethality in the dams was observed for doses > 32 µg/kg/d, but there were no adverse effects on litter size, fetal weight, or incidence of anomalies. However, as the number of surviving dams at doses > 32 µg/kg/d was small, the number of fetuses available for evaluation at the larger doses was also small and conclusions about lack of fetal effects at doses > 32 µg/kg/d are weak. Subsequently, dams were injected with 50 µg/kg/d during GD 8-12 or 13-17. Maternal toxicity, including lethality and hemorrhaging, was noted in dams exposed during both stages of gestation although the incidence and severity was less for exposure during the later period. In the dams exposed during the later period, birth occurred earlier in the day compared to controls. A reduction in litter size compared to controls was noted for exposure during both gestational periods. Pups of dams exposed during GD 13-17 had significantly reduced body weight. There was decreased fetal survival among the pups in the GD 13-17 dosing group and indication of gastrointestinal hemorrhage. Following cross-fostering to control dams, pups of dams exposed during GD 13-17 (but not GD 8-12) had decreased viability and weight gain.

The injection route of exposure is not appropriate for deriving an oral RfD. In addition, the design of this study is not amenable to deriving a useful LOAEL or NOAEL. However, this study does provide qualitative evidence of reproductive/developmental effects of CYN.

Reference Dose (RfD) derivation:

Selection of critical study – Only two repeat dose studies with specific estimates of daily dose were identified, Humpage and Falconer (2003) and de Almeida et al. (2013). The de Almeida et al. (2013) study, however, yields only a freestanding NOAEL that is an order of magnitude lower than the NOAEL from Humpage and Falconer (2003). The Humpage and Falconer (2003) study with a critical effect of increased relative kidney weight is, therefore, the more appropriate study

for the derivation of a short term RfD. The Humpage and Falconer (2003) study is also the study selected by the USEPA for its Drinking Water Health Advisory for cylindrospermopsin (USEPA, 2015b). This study yields a NOAEL of 30 µg/kg/d based on increased relative kidney weight.

Uncertainty factor (UF) analysis - A total UF of 1,000 was applied to the NOAEL based on the following individual UFs:

UF – study duration = 1

Although this was a less-than sub-chronic duration (11 week) study, it is consistent with the range of study durations applicable to the derivation of a short-term RfD (see Exposure Scenario section).

UF – LOAEL-NOAEL = 1

The study yields a reasonable estimate of the NOAEL.

UF – animal-human = 10

Standard assumption - this includes factors of 3 each for interspecies toxicokinetic and toxicodynamic variability.

UF – sensitive human populations = 10

Standard assumption – includes children as a sensitive group.

UF – database = 10

It is noted that the USEPA (2015b) applied an uncertainty factor of 3 for database insufficiency for CYN citing the same study. The study of Rogers et al. (2007) provides evidence that CYN can produce reproductive/developmental effects. However, the nature of this study does not permit the derivation of a meaningful LOAEL or NOAEL. Furthermore, there are no data that permit an assessment of potential neurological or immunologic effects. Thus, although there is evidence indicating that CYN is capable of causing reproductive/developmental effects, there is no basis for deriving a reproductive/developmental-specific RfD, and there is no basis for determining the NOAEL based on increased relative kidney weight is protective against reproductive/developmental effects. Based on this consideration as well as the lack of data on possible neurological and immunological effects, a full UF of 10 for database insufficiency appears justified.

UF - total = 1,000

Calculation of RfD

$$\begin{aligned}\text{RfD} &= \text{NOAEL} \div \text{UF-total} \\ &= 30 \text{ } \mu\text{g/kg/d} \div 1,000 \\ &= \mathbf{0.03 \text{ } \mu\text{g/kg/d}}\end{aligned}$$

Comparison to USEPA RfD - USEPA (2015d) derived an RfD of 0.1 µg/kg/d for CYN. The USEPA RfD uses the same NOAEL of 30 µg/kg from Humpage and Falconer (2003), but differs in applying a total UF of 300 rather than the value of 1,000 derived here. The basis for this difference is described above.

III. Anatoxin-a

Review of toxicological data:

Astrachan and Archer (1981) - Anatoxin-a was isolated from NRC-44-1 strain of *A. flos-aquae*. Anatoxin-a is known from previous work (Carmichael et al., 1975) to be a neurotoxin acting as a nicotinic agonist acting by stimulation followed by a depolarizing blockade to produce death with ataxia and convulsion. Based on Carmichael et al. (1975), the single dose injection (intraperitoneal) LD₅₀ is approximately 250 µg/kg in rats and mice.

Female adult Sprague-Dawley rats were exposed to anatoxin-a through either drinking water (0, 51 or 510 µg/kg/d, 20/dose group) for up to 7 weeks, or by i.p. injection (0 or 89 µg/kg/d for 21 days, 18/dose group). The oral doses are estimated to be 0.8% and 8% of the oral LD₅₀ respectively, and the intraperitoneal dose was estimated by the authors to be 25% of the i.p. LD₅₀. Based on the body weight provided in study, however, this dose appears to be 36% of the i.p. LD₅₀. Animals exposed by both routes were assessed for body weight, food consumption, behavior; gross lesions; liver, spleen, kidney weight, and histopathology of these organs; RBC and WBC count; and serum liver enzymes (cholinesterase, AP, SGTP, GGTP).

No significant differences were observed between the control and dosed groups in any of the parameters assessed. Although there was a transient increase in white blood cell count in the high dose animals at 5 weeks, this parameter was not different from the control or low dose value at 7 weeks, and the significance, if any, of this observation is unclear. Thus, 510 µg/kg/d is identified as a free-standing NOAEL from this study. The USEPA (2015e) identifies the high dose in this study as a LOAEL (rather than a NOAEL) on the basis of the transient white blood cell count. Although most of the data are presented only as summary narrative statements, the study design and results appear to be valid. However, there would be more confidence in the NOAEL if this study had identified a LOAEL.

Astrachan et al. (1980) - Teratology studies - Astrachan et al. (1980) isolated anatoxin-a from a laboratory culture of NRC-44-1 and reported that it was “essentially pure” by TLC and HPLC. Pregnant golden hamsters were dosed by i.p. injection on either GD 8-11, or 12-14. The only single daily dose was 200 µg/kg on GD 8-11. Other doses consisted of multiple daily injections (3 x 125 µg/kg = 375 µg/kg/d; and 3 x 200 µg/kg = 600 µg/kg/d) with an identical schedule for GD 8-11 and GD 12-14. Dams were sacrificed on GD 15 and the fetuses examined.

The authors do not report statistical significance for fetal resorption. However, for all dose groups and each gestation period, the percent resorption was greater than for controls – including greater than four-fold the control rate (GD 8-11, 375 µg/kg/d). Fetal weight was significantly decreased compared to controls for all dose groups for dosing on GD 8-11, and for the 375 µg/kg/d group on GD 12-14. The authors refer to “stunting” of the fetuses. This refers to reduced body weight. In addition, in one litter with maternal dosing at 375 µg/kg/d, all 10 of the fetuses had hydrocephaly. No other soft tissue or skeletal malformations were noted.

The injection route of exposure as well as the absence of a NOAEL preclude the direct use of this study in the derivation of an RfD. In addition, precise interpretation of these findings is hampered by incomplete and inexact reporting. Nonetheless, this study provides evidence that anatoxin-a exposure during gestation can have developmental effects including, at a minimum, decreased fetal weight.

Fawell and James (1994)/Fawell et al. (1999)³:

In each of the studies in this publication, Fawell and James (1994) used commercial anatoxin-a hydrochloride. The doses given below reflect adjustment to the dose as anatoxin-a (i.e., the parent molecule without the hydrochloride salt).

Single dose studies - Male CD-1 mice received anatoxin-a hydrochloride by a single intravenous injection (6 per dose group) at doses of 0, 8.2, 24.5 and 81.7 µg/kg. Mice were observed through 4 hrs post-injection and evaluated according to the Irwin protocol (a checklist of *in vivo* clinical observations). No observed effect occurred for the 8.2 or 24.5 µg/kg doses. Two animals in the 25 µg/kg dose group died and those that survived showed increased salivation, respiration and hyperactivity. No effects were observed in the low dose group (8 µg/kg anatoxin-a/kg). At 81.7 µg/kg, all animals died within 1 min of dosing with neurological symptoms consistent with a cholinergic effect. It should be noted that only a factor of approximately 3 separates the no-effect dose (relative to these parameters) from the lethal dose.

Male CD-1 mice received intravenous anatoxin-a as a single injection of 0, 24.5, 40.8, or 49 µg/kg (6/dose group). Animals were tested on the rotarod 15 min post injection. One animal died at 24.5 µg/kg, and two animals died at 40.8 µg/kg. Animals at 49 µg/kg experienced convulsions, hypersalivation, micturition, an elevated tail, and hyperactivity, and all of them died within one minute of dosing. The “majority” of animals at 24.5 µg/kg and all animals at 40.8 µg/kg had increased respiration with a duration of approximately 1 minute. Recovery of all surviving animals occurred within a few minutes. All surviving animals remained on the rotarod, indicating retention of significant neuromuscular function/coordination. Contrary to the

³Fawell et al. (1999) is a reiteration of a portion of the studies in Fawell and James (1994). The former is a journal article and the latter is a study report.

earlier intravenous injection study in this paper, 24.5 µg/kg is a LOAEL not a NOAEL here due to the apparent dose-related death at this dose.

Repeated dose studies - Two male and two female mice were dosed five times by oral gavage at each of the dose levels below. Although not stated explicitly, it appears that surviving animals were sacrificed 24 hrs after the last dose. For animals receiving 1,225 or (apparently) 2,450 µg/kg/d, dosing was on successive days. For the 6,125 and 12,250 µg/kg/d doses, dosing was every other day. All animals receiving 12,250 µg/kg/d and one female receiving 6,125 µg/kg/d died within 3 min. of dosing. At 6,125 µg/kg/d, males were hyperactive after the third dose. Body weight and food consumption were unaffected and no unusual results were observed on necropsy. No toxicity was observed at 1,225 or 2,450 µg/kg/d.

In a longer duration component of this study, mice (10 males and 10 females per dose group) received 0, 98, 490, or 2,450 µg/kg/d by gavage for 4 wks. Animals were bled without sacrifice during the final week of dosing and assessed for hematology and blood chemistry, and weight and histopathology of multiple organs was assessed at sacrifice.

One animal receiving 98 µg/kg/d was reported to have died with evidence of infection. One male receiving 490 µg/kg/d and one female receiving 2,450 µg/kg/d died within 2.5 hrs of dosing with no obvious pathology. Although there is no clear evidence linking these two deaths to the dosing, the timing relative to receiving the doses in both cases is suspicious and such a connection cannot be dismissed. There was no dose-related effect on body weight, body weight gain, or ophthalmoscope examination.

Small, but statistically significant increases in mean cell hemoglobin (Hb) (males) or mean cell Hb concentration (females) were observed. However, the increase relative to controls was small (5-6%) and the significance of this observation is unclear. A relatively large (maximum = 30%), but not statistically significant, increase in AST was observed in males at the two highest doses. However, none of the other serum liver enzymes concentrations (ALP, ALT) were remarkable, and there was no abnormal liver histopathology. There was also a relatively small (2%), but statistically significant, increase in serum Na in females. There was no significant effect on body weight or organ weight and no remarkable histopathology.

In a developmental toxicity component of this study, time-mated female CD-1 were gavage dosed once per day at 2,450 µg/kg/d (n = 10), or with sterile water (controls) during GD 6-15 (n = 12). Dams were observed for clinical signs. On GD 18, animals were sacrificed and maternal necropsy was performed. Fetal implants, live and dead fetuses, fetal weight, sex and external abnormalities were recorded. Results were only provided in narrative form with few quantitative specifics.

No maternal toxicity was observed, including no effect on body weight or weight gain. No abnormalities were noted on necropsy. Implantations, live fetuses, post-implantation losses and

sex ratio were unaffected. Mean fetal weight was “marginally” lower in the exposed group. The authors attribute this to small differences in litter size. No differences in physical abnormalities were observed between control and treated fetuses. This dose can be considered a NOAEL for developmental effects.

Although there were no clear adverse toxicological results at any of the doses, the two unexplained deaths at 490 and 2,450 µg/kg/d dictate that the clearest NOAEL is 98 µg/kg/d. A NOAEL of 98 µg/kg/d is reasonably consistent with a single dose LOAEL of 24.5 µg/kg from the i.v. injection study given the likely difference in toxicokinetics between the i.v. and gavage routes of exposure. However, it should be noted that in both studies, the LOAEL is based on lethality. This NOAEL is smaller than the freestanding NOAEL of 510 µg/kg/d in rats via drinking water from the study of Astrachan and Archer (1981).

Yavasoglu et al. (2008) - Male mice (strain not specified) were exposed by intraperitoneal injection for 7 consecutive days to 0, 50, 100, or 150 µg/kg/d of anatoxin-a. Animals were evaluated for sperm count and light microscope histopathology of the testes. There were no significant effects on body weight. However, epididymis weight decreased monotonically with significant decreases compared to controls at the two higher doses. Sperm count also decreased monotonically with all doses resulting in a significant decrease relative to controls. Histopathological changes in the testes were reported. Although counts for individual effects were not reported, the effects (including degeneration of seminiferous tubules, dissociation of spermatogenic cells with resulting sloughing of germ cells into the lumen, vacuolization of Sertoli cells, and loss of germ cells) were stated to be greater at 150 µg/kg/d than at 100 or 50 µg/kg/d. The epithelial thickness of the seminiferous tubules decreased monotonically with dose and was significantly less than controls at all doses. The injection nature of the dosing in this study precludes the quantitative use of these data in RfD derivation. However, all of the doses produced adverse effects and these results raise concerns for potential effects on male fertility with oral exposure.

RfD Derivation:

Selection of critical study – The following table summarizes the NOAELs and LOAELs for lethality from the available studies.

Study	NOAEL (µg/kg/d)	LOAEL (lethality) (µg/kg/d)	Route of Exposure
Astrachan and Archer (1981)	510 (freestanding)		Drinking water
Fawell and James (1994)/ Fawell et al. 1999)	98	490	gavage
Fawell and James (1994)/ Fawell et al. (1999)		24.5	intravenous

Anatoxin-a appears to cause lethality through a rapid onset neurologic toxicity that produces hyperactivity and convulsions. Following intravenous injection (Fawell and James, 1994), lethality occurred within 1 min. Following gavage exposure, lethality occurred in 3 min. (Fawell and James, 1994). Thus, the much larger NOAEL from Astrachan and Archer (1981) is likely due to the slower absorption and distribution following drinking water exposure (which is directly analogous to incidental ingestion while swimming). This argues that LOAELs for lethality based on intravenous or gavage routes of exposure are likely to be conservative estimates of the LOAELs for lethality by the oral (drinking water) route of exposure. However, there is insufficient information on which to estimate the ratio between the oral NOAEL and the (unobserved) LOAEL for lethality. Furthermore, although the recreational (i.e., swimming) exposure scenario utilized in this assessment (see below) is based on empirical data, it cannot account for unusual (but possible) short-term high volume ingestions while swimming. Therefore, given the potential for lethality, conservative (i.e., protective) assessment of the relationship between the oral NOAEL and the LOAELs for lethality from the other exposure routes, particularly gavage, is appropriate.

The free standing NOAEL of 2,450 $\mu\text{g/kg/d}$ (maternal exposure) from the developmental toxicity study of Fawell and James (1994)/Fawell et al. (1999) is the largest of the NOAELS. However, that NOAEL is based on a study with limited data reported, including inadequate reporting of the maternal pathology analyses that were carried out. The next largest NOAEL of 510 $\mu\text{g/kg/d}$ from the 7 week drinking water study in rats of Astrachan and Archer (1981) reflects more complete analyses and reporting. However, this dose is close to the LOAEL of 490 $\mu\text{g/kg/d}$ from the 4 week repeated dosing gavage study in mice of Fawell and James (1994)/Fawell et al. (1999). In addition, although the 510 $\mu\text{g/kg/d}$ dose in the Astrachan and Archer (1981) study is assessed here as a NOAEL, it could be argued (per USEPA, 2015e) that this is a minimal LOAEL (for increased white blood cells) rather than a NOAEL. The endpoint yielding the LOAEL from the Fawell and James (1994)/Fawell et al. (1999) study is lethality that is, presumably, dose-related. The possibility that the observed lethality is dose-related is supported by the LOAEL from the i.v. single dosing portion of this study (i.e., 24.5 $\mu\text{g/kg}$) that is also based on lethality. Thus, the free standing NOAEL (drinking water) from Astrachan and Archer (1981) differs from the LOAEL (gavage) of 490 $\mu\text{g/kg/d}$ for lethality from Fawell and James (1994)/Fawell et al. (1999) by only 16% and does not provide sufficient protection against lethality. The NOAEL of 98 $\mu\text{g/kg/d}$ from Fawell and James (1994)/Fawell et al. (1999) is a factor of five below the LOAEL (based on lethality) from that study and is, therefore identified as the preferable point of departure for RfD derivation.

Uncertainty factor (UF) analysis - A total UF of 1,000 was applied to the NOAEL based on the following individual UFs:

UF – study duration = 1

Although this was a less-than sub-chronic duration study, it appears appropriate to the relevant exposure scenarios.

UF – LOAEL-NOAEL = 1

The study yields a NOAEL.

UF – animal-human = 10

Standard assumption - this includes factors of 3 each for interspecies toxicokinetic and toxicodynamic variability.

UF – sensitive human populations = 10

Standard assumption– includes children as a sensitive group.

UF – database = 3

There is evidence from Astrachan et al. (1980) that anatoxin-a can cause developmental effects. However, that study does not yield a NOAEL. The Fawell and James (1994)/Fawell et al. (1999) study yields a NOAEL for developmental effects of 2,450 µg/kg/d (maternal dose). However, the method and data reporting for that portion of the study is inadequate. Thus, it is not entirely clear whether the NOAEL in the Fawell and James (1994)/Fawell et al. (1999) study is protective of developmental effects. In addition, there are no data that would allow an assessment of whether this NOAEL is protective of reproductive or immunotoxic effects.

UF – modifying factor = 3

The NOAEL from the critical study is less than an order of magnitude smaller than the LOAEL from the same study that reflects lethality.

UF-total = 1,000

RfD Calculation

RfD = NOAEL ÷ UF-total

= 98 µg/kg/d ÷ 1,000

= 0.098 µg/kg/d

which rounds to 0.1 µg/kg/d

Exposure Scenario - Water ingestion while swimming

Swimming is the most direct and pervasive activity that is likely to lead to exposure to hazardous algal toxins in surface water. Although there appears to be a potential for exposure to these algal toxins through inhalation and dermal exposure, direct ingestion appears to be the predominant

route of exposure while swimming (USEPA, 2016). Therefore, the exposure scenario is based on ingestion while swimming.

Water ingestion rate:

The USEPA Exposure Factors Handbook (EFH) (USEPA, 2011) provides guidance on incidental water ingestion while swimming. The rate of incidental water ingestion is greater (on both an absolute and body-weight adjusted basis) for children than for adults. For children (defined in the guidance as less than 18 years old), the guidance for the mean ingestion rate is 37 ml/event (45 min in the study used to derive this guidance) and 49 ml/hour. The upper percentile (reported as the 97th percentile of the distribution in the source study (Dufour et al., 2006) guidance for children is 90 ml/event (45 min) and 120 ml/hr. For episodic (as opposed to chronic) exposures, consistent with swimming events, the upper percentile values appear to be more appropriate. The EFH provides the value as generated by the recommended study based on the measurement time of 45 min. However, the EFH also provides a linear extrapolation of this value to a 1 hour swimming exposure in its recommendation. The length of a swimming event can be longer than the specific swimming event in the study used in the EFH, and a duration of one hour is assumed. It is recognized that the total recreational time spent near surface water used for swimming can be considerably longer than 1 hour. However, this duration of exposure refers specifically to time spent in the water.

Ingestion rate (upper percentile) for a 1 hour swimming event is 120 ml (0.12 L).

Frequency of exposure:

This is a difficult parameter to anticipate or model since it depends both on the frequency of swimming over the course of swimming season and the persistence of harmful algal blooms during that period. These appear to be highly variable. Rather than attempt to estimate this parameter quantitatively, the less-than sub-chronic to sub-chronic RfDs derived above will be assumed to be applicable as derived under the broad assumption that swimming events in water contaminated with toxins from harmful algal blooms can occur during multiple events over the course of the swimming season.

Body weight:

Because the source study used in the EFH to derive the recommended value for children's water ingestion (Dufour et al., 2006) does not specify the ages or age range of the children, the corresponding body weight cannot be derived directly from the recommended ingestion rate. The EFH provides recommendations for body weights for the entire range of childhood. Since hazardous algal blooms occur in natural waters (as opposed to, e.g., pools), it is assumed that an exposure scenario envisioning multiple swimming events would be most applicable to children who can swim and/or participate in water activities by themselves. The youngest age range addressed by the EFH that corresponds to this criterion is 6 to <11 years old. The mean body weight for this age group is given as 31.8 kg.

Body weight (ages 6 to <11 years) is 31.8 kg.

Calculation of recommended target concentrations of hazardous algal toxins

Equation

The recommended target water concentration of a toxin is given as:

$$C = \frac{\text{RfD} \times \text{BW}}{I}$$

Where:

C = the concentration of the toxin in the swimming water (µg/L, ppb)

RfD = the Reference Dose for the specific toxin (µg/kg-body wt/day)

BW = the assumed body weight of the child (31.8 kg)

I = the ingestion rate of swimming water (0.12 L/day)

Recommended target concentrations

Microcystin-LR

$$C = (0.01 \text{ µg/kg/d} \times 31.8 \text{ kg}) / 0.12 \text{ L/day} = 2.65 \text{ µg/L}$$

This is rounded to 3 µg/L.

Cylindrospermopsin

$$C = (0.03 \text{ µg/kg/d} \times 31.8 \text{ kg}) / 0.12 \text{ L/day} = 7.95 \text{ µg/L}$$

This is rounded to 8 µg/L.

Anatoxin-a

$$C = (0.1 \text{ µg/kg/d} \times 31.8 \text{ kg}) / 0.12 \text{ L/day} = 26.5 \text{ µg/L}$$

This is rounded to 27 µg/L.

Discussion

There are numerous uncertainties related to the recommended values for these hazardous algal toxins. The literature appropriate for consideration in RfD derivation was quite limited and in most cases only a single applicable study was available. The application of uncertainty factors of 1,000 (cylindrospermopsin and microcystin) and 1,000 (anatoxin-a) reflects the incompleteness of the databases for these toxins as well as the lack of clarity about the significance of reported outcomes. The specification of the likely exposure scenarios is highly uncertain due to lack of information about the nature, duration, and frequency of the actual exposures, and the likely duration of the toxins in any given waterbody. In particular, the density of blue-green algae at the surface of a body of water is subject to rapid change resulting from wind conditions. The uneven pattern of algal growth and the rapid shift in bloom density make obtaining a representative sample difficult.

Given these multiple uncertainties, the recommended water concentrations given here are intended to be protective of a range of exposures and are probably highly conservative (i.e., protective) for the most likely exposures. Nonetheless, the extent of this conservatism is not known. The uncertainty in the risk estimates as well as the inherent uncertainty in the temporal variability of the toxins in any given waterbody should be taken into account when considering advice to the public regarding recreation in affected waterbodies.

These recommendations do not address the risk to pets, livestock and wild fauna, nor do they address the risk associated with consuming fish from affected waters or the combined risk from swimming and fish consumption.

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