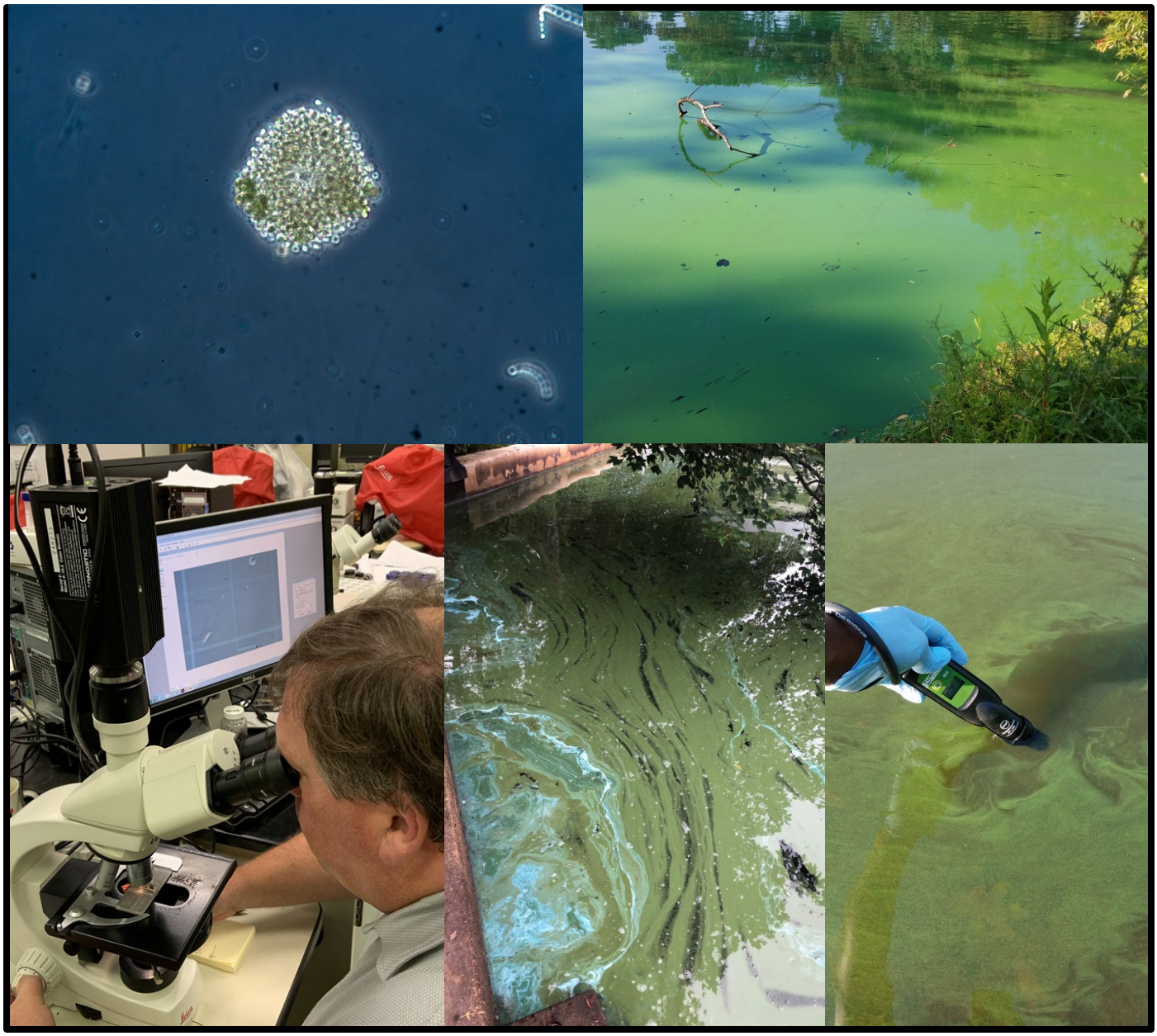
NJ Department of Environmental Protection
Division of Water Monitoring and Standards
Bureau of Freshwater & Biological Monitoring

2020 Cyanobacterial Harmful Algal Bloom (HAB) Freshwater Recreational Response Strategy



June, 2020

ACKNOWLEDGEMENTS

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 - Bureau of Freshwater & Biological Monitoring, Bureau of Marine Water Monitoring, Bureau of Environmental Analysis, Restoration and Standards and Director's Office, Water Resource Management Assistant Commissioner's Office, Division of Science and Research, Division of Water Supply and Geoscience, State Park Service, Division of Fish & Wildlife, Office of Quality Assurance, and Water Compliance and Enforcement; 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.

Contributors to the development of this document include: Victor Poretti (lead), Leslie McGeorge, Tom Miller, Dean Bryson, Alena Baldwin-Brown, Gloria Post, Rob Newby, Leigh Lager, and Robert Schuster. The authors wish to thank Katrina Angarone, Michele Putnam, Bruce Friedman and Gary Buchanan for their reviews and support in the development of this Strategy.

If there are any questions or comments on the HAB Strategy, please provide them to: njcyanohabs@dep.nj.gov.

TABLE OF CONTENTS

Acronym List

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

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

DoA - New Jersey Department of Agriculture

DOH – New Jersey Department of Health

DSR - DEP Division of Science and Research

DWMS - DEP Division of Water Monitoring and Standards

DWSG -DEP Division of Water Supply and Geoscience

EOH - 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

LHA - Local Health Authorities

NLA - National Lakes Assessment, USEPA

OHHABS - CDC One Health Harmful Algal Bloom System

qPCR - quantitative Polymerase Chain Reaction

PRB – Public Recreational Bathing facility

UAV – Unmanned Aerial Vehicle

UCMR - Unregulated Contaminant Monitoring Rule, USEPA

USEPA - United States 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 the New Jersey Cyanobacterial Harmful Algal Bloom (HAB)* Response Strategy (Response Strategy) is to provide a unified statewide approach to respond 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 of the Response Strategy is the protection of human health, it provides some information and recommendations regarding exposure and prevention of potential impacts to domestic animals (pets), livestock, and wildlife, as well. The Response Strategy is designed to identify:

- Entities responsible for response and actions
- Recreational risk thresholds and appropriate responses to protect public health and safety
- Acceptable parameters and methods for assessing risk
- Appropriate monitoring and analysis to identify cyanobacteria, enumerate cells and determine concentrations of cyanotoxins, and
- HAB Alert Levels, recommended advisory language and other related communication mechanisms.

The scope of the Response Strategy is for freshwater lakes, ponds, rivers and streams with potential public access, recreational use, public recreational bathing facilities as defined in N.J.A.C. 8:26, 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 HAB concerns are addressed by the Department of Environmental Protection's (DEP's) Division of Water Supply & Geoscience (DWSG), as detailed in a specific drinking water response protocol and guidance. The response protocol outlines the communication during a HAB/cyanotoxin event, including the coordination between the Division of Water Monitoring and Standards (DWMS), the Division of Water Supply and Geoscience (DWSG), and the water system. DWSG requires water systems that are at risk for a HAB to plan for such events as part of their Emergency Response Plan and is also working with these systems to develop management plans based on the guidance from the 2015 USEPA "Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water " and subsequent updates. For more information on drinking water and HABs, see the DWSG website: http://www.nj.gov/dep/watersupply/.

New Jersey released its first Response Strategy in 2017 and since then has continued to enhance all aspects of its approaches including, response monitoring, testing, notification methods and research. HAB events from 2017-2020 are described at https://www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. In November of 2019, Governor Phil Murphy announced a Harmful Algal Blooms (HABs) Initiative to comprehensively address these blooms in the State. The Initiative has three main components: to reduce and prevent future harmful algal blooms; to enhance HAB science, and build monitoring, testing and data management response capacity; and to improve communication, including HAB website enhancements and interactive mapping and reporting. Details of this Initiative can be found at: https://www.state.nj.us/dep/hab/docs/HABs_factsheet_11.14.19rev2.pdf

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

A. Agency Responsibilities

An interagency HAB Workgroup was formed in 2016, consisting of representatives from the DEP, the New Jersey Department of Health (DOH), and the New Jersey Department of Agriculture (DoA) to discuss and collaborate on HAB issues, including: Response Strategy development, monitoring, laboratory analysis, risk thresholds, advisories, research and communication. Following development and release of the initial version of this Response Strategy in 2017, the Workgroup has met periodically after each HAB season to enhance the Response Strategy based on New Jersey's experience responding to HABs, the State's HAB and water quality data, updated information on HAB science, evaluation of other States' HAB strategies, available federal guidance, and New Jersey HAB partner input. Appendix A contains a list of the members of the Workgroup and their contact information and provides a link to local/county Health Department emergency contact information for this Response Strategy.

The following are the responsibilities of each state agency tasked with contributing to this Response Strategy.

NJ Department of Environmental Protection (DEP)

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

- Develop, maintain and enhance monitoring and analysis capacity for cyanobacteria/cyanotoxins.
- Perform surveillance and screening for freshwater HABs including field sampling, monitoring, and reconnaissance work on lakes, rivers and streams as required.
- Oversee HAB information dissemination on DWMS/BFBM website https://www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html, including HAB events and data. Develop and maintain HAB Interactive Mapping and Communication System.
- Provide content for HAB information dissemination and outreach, including production and maintenance of general HAB information, outreach materials and fact sheets on DWMS/BFBM website. Work in cooperation with DWMS Director's Office to provide content for DEP general HAB website https://www.state.nj.us/dep/hab/
- Work with other divisions and programs throughout DEP to maintain DEP general HAB website.
- Coordinate with DEP State Park Service, DEP Division of Fish and Wildlife and NJ Department of Health regarding outreach material development and dissemination.
- Notify New York State Department of Environmental Conservation/ Division of Water regarding HABs occurring in waterbodies that span the NY/NJ boarder including, Greenwood Lake, West Milford, Passaic Co.;Lake Tappan (reservoir), River Vale & Old Tappan, Bergen Co.; Potake Pond, Ringwood Boro, Passaic Co.; Ramapo R., Mahwah Twp, Bergen Co., Mahwah R., Mahwah Twp, Bergen Co.; Wallkill R., Wantage Twp., Sussex Co.)
- Coordinate exchange of data and advisory communication with New York State Department of Environmental Conservation/ Division of Water.
- Develop and maintain HAB reporting procedures. Collect and review reports following submissions and determine who should be contacted for follow-up.
- Upon notification of a suspected HAB incident (Algal Bloom), DEP's BFBM will serve as the lead to investigate and coordinate responses consistent with Section 4 of this document, as applicable to the event. 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.

- Investigation and analysis will be designed to quantify cyanobacteria levels above a cell count of 20,000 cells/ml and toxins above NJ Guidance Levels.
- Coordinate additional field surveillance and monitoring at Public Recreational Bathing facilities (PRB) when Alert level is reached upon a cell count of 40,000 – 80,000 cells/ml.
- Monitor and analyze suspected and confirmed blooms. Depending on waterbody jurisdiction and use, may include direct monitoring and analysis by BFBM and/or coordination and guidance for partner surveillance and monitoring and, on occasion, analysis of blooms.
- Coordinate implementation of Response Strategy with other New Jersey State, local and federal agencies.
- Coordinate investigation and response with appropriate partners. Internal DEP partners include the program areas of Division of Fish and Wildlife, State Parks Service, Water Compliance & Enforcement, Water Supply & Geoscience, and external partners such as county and/or local health and parks departments.
- Develop and maintain Standard Operating Procedures (SOPs) for performing field screening measurements, sampling, and laboratory analyses for HAB response. Develop training for others to use SOPs.
- Coordinate with New Jersey DOH for information dissemination and outreach to local health departments and the public regarding the potential effects of HABs.
- Coordinate with DEP's Communication Center to forward reports of suspected HAB incidents the Center receives to the BFBM.
- Provide analysis results to partners with advisory recommendations based on established New Jersey Health Advisory Guidance Levels, Alert tiers and recreational use.
- Provide analysis results and advisory recommendations to DOH and local health agencies related to Public Recreational Bathing (PRB) facilities to inform DOH and local health agencies of Alert Level actions at PRBs.
- With DEP Division of Science and Research, co-chair HAB Research Committee. Report on recommendations of the Committee, provide guidance and participate in research efforts to meet HAB information needs.
- With DEP Office of Information Technology and other DEP programs, participate in the HAB Detection and Monitoring – Unmanned Aerial Vehicle (UAV) Operations Committee and make recommendations for UAV use in HAB response. Explore uses of BFBM's current and future UAVs in screening for HABs.
- Provide training in proper sample collection and phycocyanin field meter use to partners as needed.

DEP State Park Service

- Provide general HAB outreach materials such as posters and pamphlets to Park users.
- Provide assistance in conducting HAB field surveillance, field screening and sample collection to support HAB response at State Park Lakes.
- Visually monitor State Park waterbodies for HAB development. Physically monitor HABs using equipment such as test strips and phycocyanin field meters when such equipment and training is provided.
- Contact BFBM and DOH when suspected HABs are observed at a public recreational bathing facility (PRB), or in other recreational areas, for sample collection and analysis.
- Post advisories at State Park lakes using guidelines in this document (Section 5). Also, include posts on Parks Facebook page and website.
- After initial response and issuance of advisory, it is the responsibility of State Parks Service to communicate any change in status to BFBM and DOH throughout the HAB event, until the advisory is lifted. Provide outreach to the public about HABs.
- Coordinate with BFBM and DOH on additional field surveillance and monitoring at Public Recreational Bathing facilities when Alert level is reached upon a cell count of 40,000 – 80,000 cells/ml.
- Contribute to the management of State Park lakes for the prevention of HABs. Prepare and implement Lakes Management Plans to minimize HABs.

DEP Division of Science and Research (DSR)

- Provide HAB scientific and technical support concerning human health exposure and impacts.
- Provide scientific support in cyanobacterial identification and enumeration, and toxin analysis.
- Provide technical consultation regarding bloom response.
- Provide scientific basis for revisions of guidelines/thresholds for cyanobacteria and related toxins for recreational risk using the best available science.
- With BFBM and the Research Committee of the HAB Workgroup, research new developments in HAB monitoring, analysis, prediction, treatment and impacts.
- With BFBM, co-chair HAB Research Committee. Report recommendations of Committee and provide guidance.

DEP Division of Water Supply and Geoscience (DWSG)

- Manage Emergency Response/HAB Management Plans.
- Focus on prevention, response, treatment, and follow-up of drinking water contamination as it applies to cyanobacterial HABs and toxins.
- Coordinate with DWMS/BFBM regarding source water HABs, including reservoirs used for both drinking water and recreational activities. Provide DWMS/BFBM with information on whether source waters are being used for water supply at time of HAB event, and if so, if direct or indirect source of drinking water.
- Largely external to this Recreational Response Strategy, coordinate appropriate response to HAB events with drinking water systems, including but not limited to:
    - Discuss with the system the potential for impact based on the location of the bloom in relation to the surface water intake.
    - Timely and appropriate sampling, reporting, and communication of results with relevant agencies.
    - Appropriate alteration of treatment techniques.
    - Identification of and/or approval of use of alternate supply, where feasible.
    - Interact with and report to appropriate emergency response officials as set forth in an incident command structure.
- Provide periodic updates on regulatory water system cyanotoxin monitoring data (i.e., Unregulated Contaminant Monitoring Rule 4) at interagency HAB Workgroup meetings.

DEP Division of Fish and Wildlife

- Provide general HAB outreach materials such as posters and pamphlets to fishing community and Wildlife Management Area (WMA) visitors.
- Visually monitor waterbodies during scheduled field sampling activities for suspected HAB development. Contact BFBM when blooms are sighted for sample collection and analysis.
- Post advisories at Wildlife Management Area (WMA) lakes using guidelines in this document (Section 5). Also, include posts on Fish and Wildlife Facebook page and website.
- After initial response and issuance of advisory, communicate any change in status to BFBM throughout the HAB event, until the advisory is lifted.
- Request, as needed, BFBM's assistance with HAB monitoring of fish stocked waterbodies.
- Provide a link to the CyanoHAB Events website (https://www.state.nj.us/dep/wms/bfbm/cyanoHABevents.html) on an appropriate DFW web page to provide the fishing public current status of HAB events on NJ waterbodies.
- Report fish kills to BFBM prior to, during or shortly after known HAB events which may be potentially linked to these events.
- When requested, DFW will perform necropsy and/or submit liver tissue samples from fish and wildlife cases with suspected mortality from HABs to an appropriate lab for confirmation of tissue toxins.
- Contribute to the management of WMA lakes for the prevention of HABs and prepare and implement Lakes Management Plans to minimize HABs.

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

- Provide assistance in conducting HAB field surveillance, field screening and sample collection to support HAB response.
- With DEP Office of Information Technology, participate in the HAB Detection and Monitoring - UAV Drone Operations Committee and make recommendation for UAV use in HAB response. Provide assistance as needed to BFBM in UAV field applications for HAB screening.

DEP Emergency Management Program

- Maintain the functionality of the DEP Hotline/Communication Center to gather and share incident reports involving a suspected HABs 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 (CEOHS)

- Enforce DOH regulation, New Jersey State Sanitary Code Chapter IX Public Recreational Bathing N.J.A.C. 8:26.
- Advise and make appropriate recommendations regarding inspected or permitted freshwater, public recreational bathing facilities (PRBs), including New Jersey State Park bathing facilities.
- Maintain and provide to DEP (for response and reporting purposes) a list of all State licensed freshwater PRBs with waterbody names, locations (coordinates, municipalities and counties) and local health department emergency contact information.
- Work with DEP to develop a PRB Notification System that, for the first time, will include freshwater beaches Offer technical assistance and consult with DEP regarding HAB human 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/or inspect PRBs within their jurisdictions.
- Notify local health authorities of required actions to be taken at PRBs when HAB Notice or Advisories/Beach Closures are warranted.
- Confirm advisories have been issued.
- Coordinate additional field surveillance and monitoring at Public Recreational Bathing, when Alert level is reached at a cell count of 40,000 – 80,000 cells/ml, with BFBM and local health authorities.
- Contribute to development of HAB Alert Levels in consultation with DEP.
- Provide information to the public regarding HAB awareness, including use of DOH websites.
- Provide outreach to the public about the health effects of HABs, in conjunction with DEP, including assistance with distribution of HABs-related outreach materials https://www.state.nj.us/health/ceohs/documents/phss/hab_resource_list.pdf

Communicable Disease Service (CDS)

- Review and monitor human illness reports to determine if illnesses may be associated with HAB exposure.
- Public Health Veterinarian to review pet (e.g., dog) illness reports to determine if symptoms consistent with exposure to HABs or confirmed to be associated with HAB exposure.
- Maintain the Waterborne Illness webpage: https://www.nj.gov/health/cd/, that features HAB-related information and awareness material for the public.
- Provide outreach to the public about the health effects of HABs, in conjunction with DEP, including assistance with distribution of HABs-related outreach materials.

Local Health Authorities (LHA)

- Conduct inspections of PRB's where a suspected HAB has been identified and/or confirmed.
- Enforce DOH regulation, New Jersey State Sanitary Code Chapter IX Public Recreational Bathing N.J.A.C. 8:26.
- Provide confirmation of advisory posting or other actions taken for any PRB which was closed to recreational bathing to CEHOS at prb@doh.nj.gov .
- Coordinate with BFBM and DOH additional field surveillance and monitoring at Public Recreational Bathing facilities when Alert level is reached at a cell count of 40,000 – 80,000 cells/ml.
- Provide information to the public regarding HAB awareness.
- Provide outreach to the public about the health effects of HABs, in conjunction with DEP and DOH including assistance with distribution of HABs-related outreach materials.

New Jersey Department of Agriculture

Division of Animal Health/ New Jersey Animal Emergency Response

- Review and monitor livestock illness reports to determine if illnesses may be associated with HAB exposure.
- Receive and review notifications by DEP of HAB occurrences in waterbodies that may affect livestock.
- Notify BFBM of any reports of potential livestock illnesses which may be related to HABs 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 lifted.

2. BACKGROUND

A. Cyanobacteria

Cyanobacteria are a type of bacteria capable of photosynthesis. Although they are not true algae, they were often referred to as "blue-green algae" in the past. Cyanobacteria can discolor the waters and 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 animals. Although problems related to cyanobacteria most often occur in freshwaters (lakes and streams), cyanobacteria can also be found in coastal 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 (cyanotoxins). DEP defines a HAB as a density of identified cyanobacterial cells of 20,000 cells/ml or higher. HABs often occur under suitable environmental conditions of light, temperature, nutrient enrichment, and calm water. These blooms can result in a thick coating or mat on the surface of a waterbody, frequently in summer or fall, but blooms can occur year-round. A general overview fact sheet about Cyanobacterial Harmful Algal Blooms (HABs) and a technical fact sheet related to recreational exposure and health effects are available at: http://www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html.

B. Cyanobacterial Blooms and Toxins

Cyanobacterial blooms may vary in species composition, residence time, the cyanotoxins they produce, and the associated 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 of HABs can be waterbody-wide, or localized near the shoreline, shallows or areas affected by flows or the influx of nutrients.

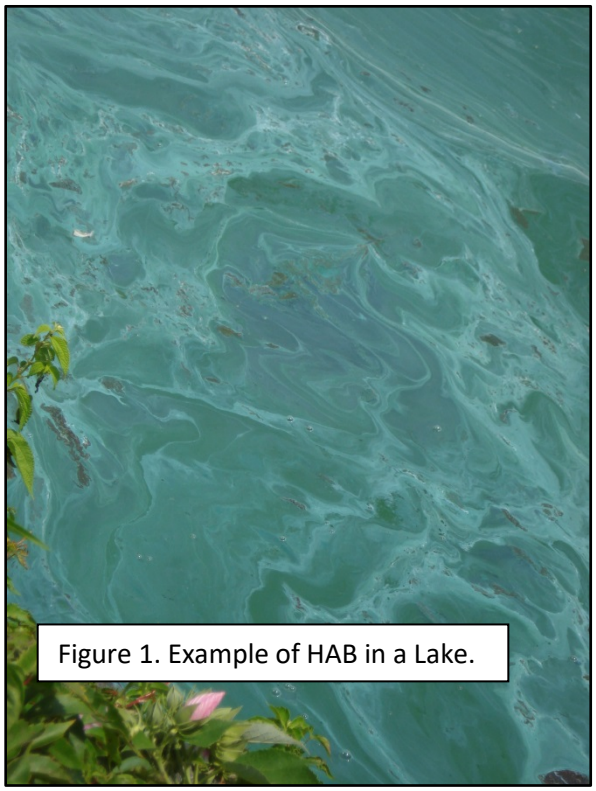


Figure 1. Example of HAB in a Lake.

Cyanobacteria may maintain a position at a particular depth or may be found throughout the water column where light penetrates (e.g. *Planktothrix, Cylindospermopsis*). Some 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 of more than one meter.

3. HUMAN HEALTH RECREATIONAL RISK THRESHOLDS

A. Human Health Impacts from Exposure to Cyanobacteria and Toxins

Exposures to cyanobacteria and cyanotoxins during recreational activities may potentially occur through oral ingestion (swallowing), skin absorption, and inhalation. Oral exposure may occur from accidental or deliberate ingestion of water. Dermal exposure occurs 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. However, such inhalation exposure is much lower than ingestion exposure that can occur from immersion during recreational activities, such as swimming.

Adverse health effects from recreational exposure to cyanobacterial cells and cyanotoxins can range from a mild skin rash to serious illness. Acute illnesses caused by exposure to cyanotoxins have been reported, and exposure to very high levels of toxins is potentially fatal.

Allergic–like reactions (e.g., rhinitis, asthma, eczema, and conjunctivitis), flu–like symptoms, gastroenteritis, respiratory irritation, skin rashes, and eye irritation can occur through primary recreational exposure to cyanobacterial cells. These effects are caused by components of the cells that are present regardless of whether the cells are producing cyanotoxins. Allergic or irritative skin reactions of varying severity have been reported from recreational exposures where the presence of freshwater cyanobacteria, such as *Dolichospermum* (Figure 2*), Aphanizomenon, Nodularia*, and *Oscillatoria* endotoxins have been confirmed. Skin and eye irritation, from exposure during swimming, have been related to the cyanobacterial cells and dermal toxins produced by cyanobacteria.

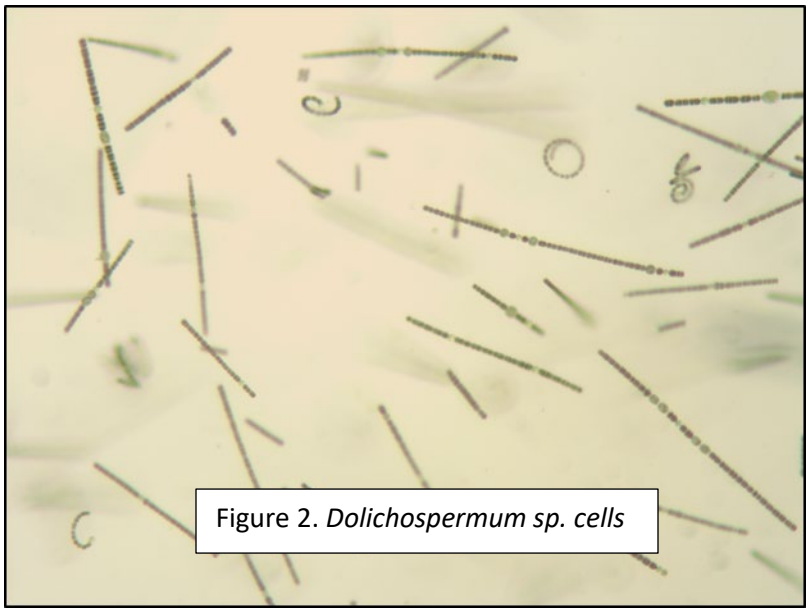


Figure 2. *Dolichospermum sp. cells*

In addition, cyanotoxins such as microcystins and anatoxin-a can cause gastrointestinal illness, liver disease, neurological effects, and skin reactions. While cyanotoxins are not classified as carcinogens by USEPA, studies in laboratory animals and cultured cells suggest that microcystin can cause liver tumors and microcystin and nodularin promote the growth of existing liver tumors. 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. (Note: 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.) (USEPA's HABs website: (https://www.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-water)

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. (USEPA's HABs website: (https://www.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-water)

It should be noted that many types of toxins can be produced by HABs, and that most of these toxins cannot be measured by HAB response organizations. DEP, like most such organizations, routinely measures for microcystins – the most common group of cyanotoxins.

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 HABs website : https://www.epa.gov/cyanohabs)

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

B. Human and Animal Exposure and Treatment - Cyanobacteria and Toxins

Currently, New Jersey does not have specific or separate toxicological assessments for livestock or pets. Development of these values may be considered in the future. Pets, livestock, and wildlife have all had well documented adverse health outcomes when exposed to cyanobacteria and cyanotoxins. Pets, particularly dogs, may unknowingly ingest cyanobacteria or their toxins by either directly drinking water or by licking their fur after recreating. Therefore, it is best for pets and livestock to avoid any visible blooms.

The Center for Disease Control (CDC) states that if you or your pet come in contact with a cyanobacteria bloom, you should wash yourself and your pet thoroughly with fresh water. If you swallow water from a waterbody where a harmful algae bloom is present, call your health care provider or a Poison Center. If your pet drinks water from a waterbody where a harmful algae bloom is present, call a veterinarian. Also 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.

C. Cyanobacteria and Cyanotoxin Risk Thresholds for Recreational Waters

In 2019, EPA developed recommended recreational ambient water quality criteria/ swimming advisories for two types of cyanotoxins - microcystins and cylindrospermopsin. (https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf), while the World Health Organization (WHO) http://www.who.int/water_sanitation_health/publications/srwe1/en/ (Appendix D), and a number of states have derived their own "action levels" or health advisory guidelines based on cyanobacteria cell counts and/or concentrations of the more toxic and most commonly occurring cyanotoxins.

New Jersey has developed State guidance levels for cyanobacterial cell counts and for three of the most commonly observed cyanotoxins (microcystins, cylindrospermopsin and anatoxin-a) discussed below. DWMS/BFBM's laboratory has the capability to enumerate and provide taxonomic identification of cyanobacterial cells, it is certified in microcystins analysis, and uses approved methodology to reliably measure other toxins at concentrations below the specified threshold limit.

D. Cyanobacterial and Cyanotoxin Health Advisory Guidance Levels

DEP, with the support of the HAB Workgroup, has developed health advisory guidance levels and a matrix of action levels for the protection of human health from the effects of exposure to different levels of cell counts and toxin concentrations. See Table 2 for this matrix which describes the various health effects risk indices and associated Health Advisory Guidance Levels.

- **Alert Levels - Cyanobacterial cell count bases**

Exposure to cyanobacteria cells themselves, whether or not the bloom is actively producing cyanotoxins, may cause allergenic and/or irritative effects to a portion of an exposed population. These effects are caused by endotoxins (mainly from components of the cyanobacterial cell wall) rather than cyanotoxins. It has been established that some sensitive individuals have adverse allergenic/irritative responses from

exposure to cyanobacterial cells at concentrations as low as 5,000 cells/ml (USEPA, 2019).

NJ Watch: Health Advisory Guidance Level- DEP defines a HAB as a density of identified cyanobacterial cells of 20,000 cells/ml or higher. This definition is supported in the scientific literature and is widely accepted by many organizations (Loftin et al, 2008).

WHO cyanobacterial cell count guidance indicates that exposure to cyanobacteria in concentrations between 20,000 cells/ml and 100,000 cells/ml can result in a moderate probability of acute health effects (WHO, 2009).

When a HAB is present, based on cyanobacterial cell counts of at least 20,000 cells/ml (but less than 80,000 cells/ml, and with cyanotoxin levels below the NJ advisory guidance levels – see below), Watch advisories will be posted to notify the public that a HAB is present and to protect against the probability of potential allergic and/or irritative health effects from recreational exposure to the cells themselves.

If the cyanobacterial cell count is between 20,000 - 80,000 cells/ml (and toxins are below NJ advisory guidance levels) in an area where primary recreational contact is likely to occur, local authorities will be notified to surveil and monitor the area for changes in the bloom condition and notify the DEP if such changes occur. Frequency will be determined on a case by case basis, based on such factors as recreational use, extent of bloom, resources available, and seasonal variability.

At PRBs, an Alert for more frequent monitoring will occur when the cell count is between 40,000 - 80,000 cells/ml. If the intensity of the bloom increases as determined by visual observations or other screening methods (such as meter phycocyanin measurements or toxin "strip tests" with secondary confirmation), DEP should be notified to perform sampling and laboratory analysis to ensure the cell count has not increased or that toxin production is not above Health Advisory Guidance Levels for primary contact at a PRB which would require a beach closure.

NJ Advisory: Health Advisory Guidance Levels – While exposure to cyanobacterial cells that are not producing toxins can result in the allergenic-like, flu-like and irritative effects discussed above, more serious health effects can result from exposure to cyanotoxins. Blooms may begin producing toxins at any time during an active HAB.

DEP conducted an evaluation of NJ-specific HAB data to determine if there was a level of cyanobacterial cell density that is associated with an appreciable likelihood that a bloom will produce toxins at levels above the NJ toxin thresholds. These data were collected from 2017 to 2019 and included 935 paired cell count and microcystin results. This DEP data set was available due to the large number of HAB samples collected over the three-year period during which the NJ HAB Response Strategy was being implemented. All these data were then managed and entered into a new DEP NJ HAB database which became available in early 2020.

The HAB data were evaluated by analyzing the percentage of samples exceeding the NJ advisory guidance level for microcystins (the most common group of cyanotoxins) of 3 µg/L for various ranges of cyanobacteria cell counts. Cell count ranges were used to allow for a sufficient number of samples for statistical analysis within each range. The data shows a substantial increase in the likelihood of toxin levels above the NJ guidelines when cell counts exceeded 80,000 cells/ml (See Figure 3).

Figure 3. Percent of Cyanobacteria Bloom Response Samples Exceeding Microcystin Health Advisory Guidance Level of 3 µg/L in 2017-2019 Data.

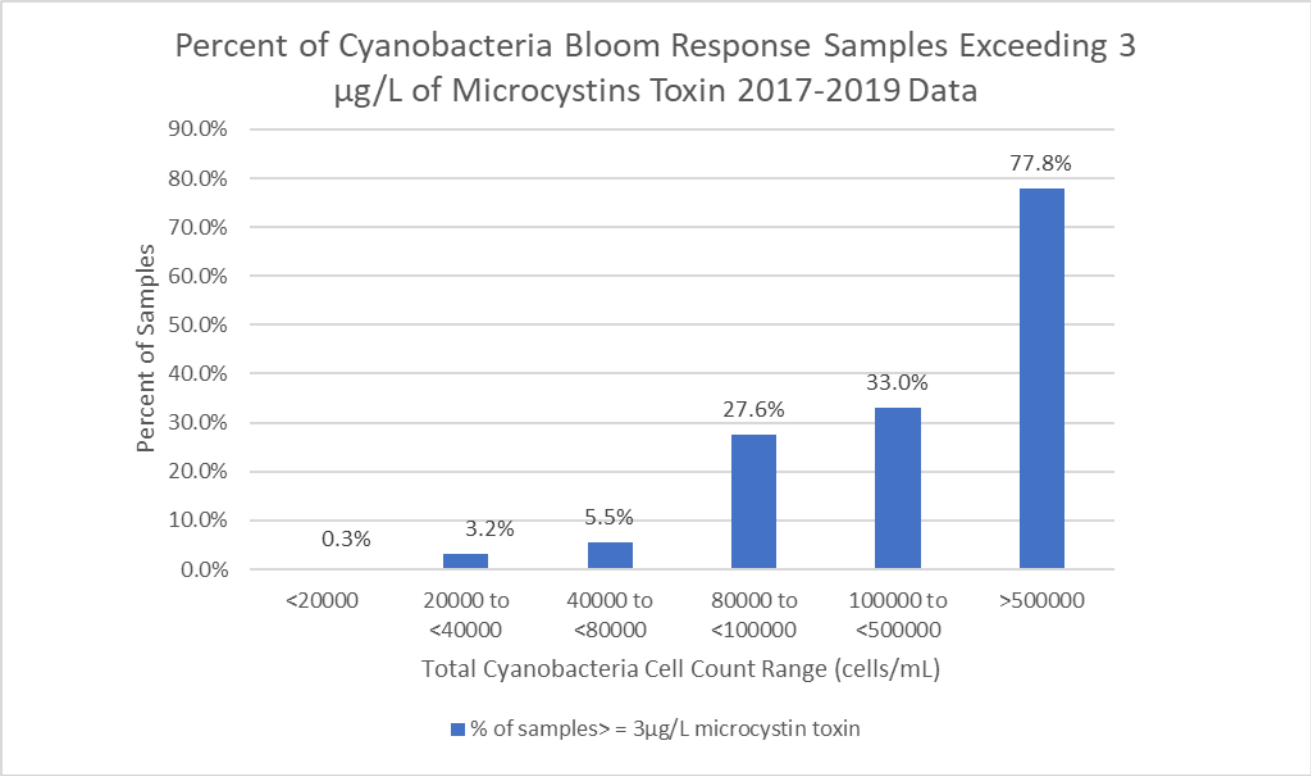


Figure 4. Linear Regression of the Log of Cell Count versus Log of Microcystin Toxin Concentration (µg/L) in 2017-2019 data.

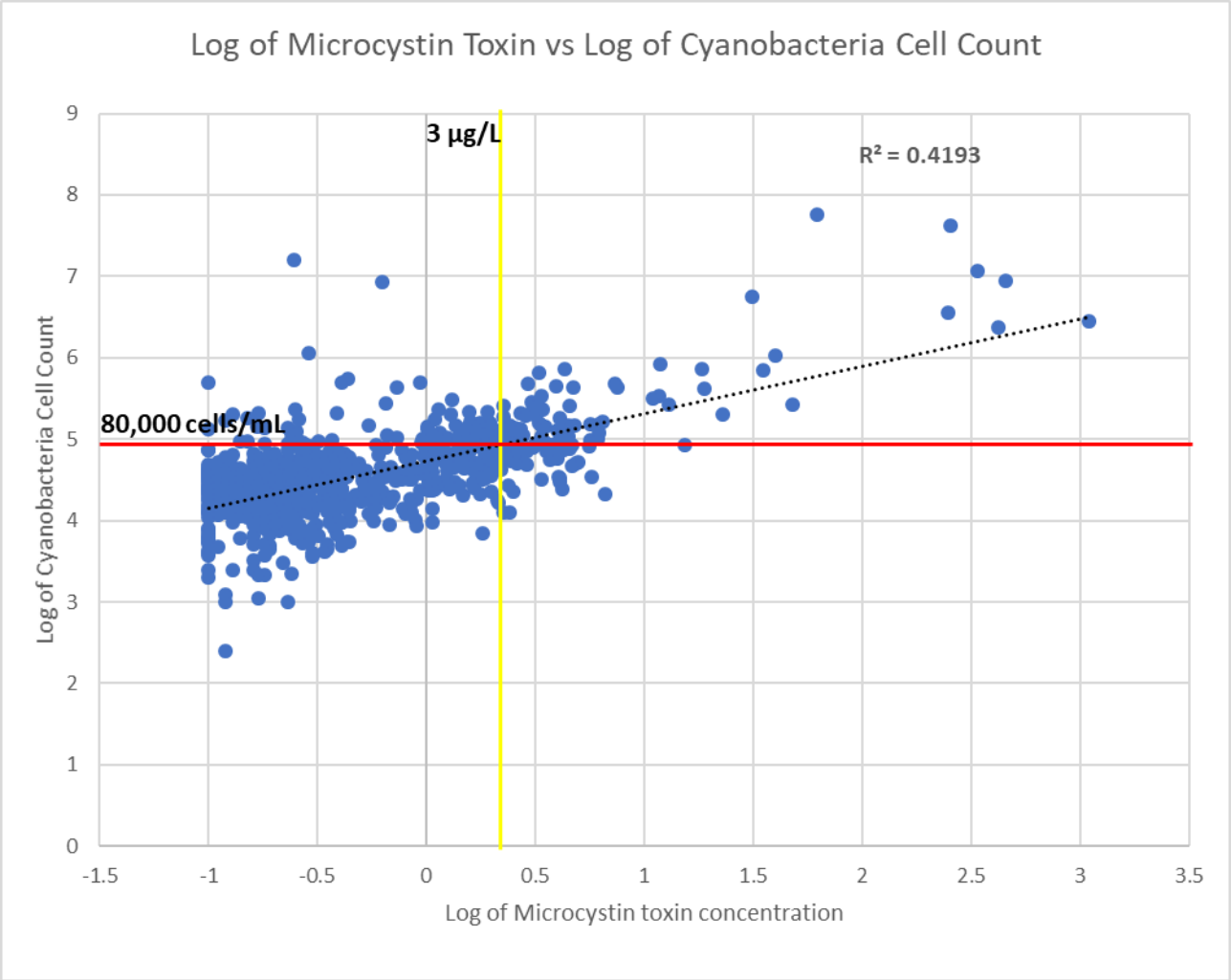


Figure 4 is the linear regression of the log of the cell counts versus the log of the toxin concentration. A log scale was used to be able to cover the large range in the cell count data, up to 56,000,000 cells/ml. This figure shows that the 3 µg/L microcystin threshold is more likely to be exceeded when the cell count is greater than 80,000 cells/ml. The yellow and red lines are where approximately 80,000 cells/ ml and 3 ug/L of microcystin toxin intersect, and shows the greater likelihood of exceeding 3 µg/L of microcystin when the cell density is above 80,000 cells/ml.

Additionally, advanced logistic regressions were also performed on these data to evaluate relationships between the probability of exceeding the microcystin health advisory guidance level of 3 µg/L and cell count. Overall, the probability of exceeding the microcystin health advisory guidance level increased as the cell count (cells per ml) increased for all subsets of the dataset.

Therefore, to ensure the protection of public recreational health, an advisory and beach closures are recommended when cell counts are > 80,000 cells/ml due to the increased probability that toxins in excess of 3 µg/L of microcystins could be produced. This threshold is also protective for the increased risk from the cells themselves at these levels, as well as for the increased probability of toxin production

to levels exceeding the health advisory guidance level at any point during the duration of the HAB. It should be noted that many types of toxins can be produced by HABs, and that most of these toxins cannot be measured by HAB response organizations. DEP, like most such organizations, routinely measures for microcystins – the most common group of cyanotoxins.

Health agencies have the authority to close public recreational bathing (PRB) facilities under the New Jersey State Sanitary Code, Chapter IX - Public Recreational Bathing, N.J.A.C. 8:26-8.5 "Criteria for closure of a public recreational bathing facility." Under these criteria, any conditions which pose an immediate health or safety hazard shall be grounds for closure of bathing and swimming activities. The DOH may use Alert Levels and Health Advisory Guidance Levels defined in this Strategy to interpret an immediate health hazard.

- **Health advisory guidance levels for individual cyanotoxins - Basis for Advisory (including Beach Closures), Warning and Danger Action Levels**

The DEP Division of Science and Research (DSR) recently reviewed the basis for health advisory guidance levels for three cyanotoxins (microcystins, cylindrospermopsin, anatoxin-a) that it developed in 2017. The basis for these recreational advisory guidance levels, including the toxicological basis (Reference Doses) and exposure assumptions, is provided in Appendix E - Basis for Health Advisory Guidelines. It is important to note that 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.

Based on the information presented in Appendix E, DEP recommends the following guidance values for recreational exposure to individual cyanotoxins:

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

An advisory and/or beach closure will be recommended when toxins are present at or above these levels regardless of cyanobacterial cell concentration. If microcystin levels are present at levels associated with high (≥20 µg/L) or very high (≥2000 µg/L) toxin levels, additional advice and actions will be warranted as per the Alert Level Summary table (See Section 5, Table 2).

4. INVESTIGATION AND RESPONSE TO HARMFUL ALGAL BLOOMS IN RECREATIONAL WATERS

A. Initial HAB Report

A cyanobacterial bloom may often be visible as a blue-green, green, yellow-green, brown, pink or possibly red discoloration on the water surface. The visible bloom may blow with the wind or move with water flow, and may accumulates in shallow areas, forming very dense scum. 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 algal blooms are due to common green algae and not cyanobacteria. It is also important to note that cyanobacteria blooms do not always produce cyanotoxins.

If you observe what you think might be a HAB in a pond, lake, or stream, submit the report via smartphone or PC using the NJDEP HAB Interactive Map Reporting and Communication System (HAB System). If a smartphone or PC is not available, call the DEP Hotline (1-877-WARNDEP) to report it.

The NJDEP HAB System will allow the reporting of suspected HABs, as well as facilitate the provision of additional information such as site coordinates and photos. This tool is intended to gather and display reports and sampling for all freshwaters where a HAB is suspected. The reports will be immediately available to DWMS/BFBM staff who will determine the entities and partners who may be available to be contacted for follow-up. Partners could include: local health departments, state and local park authorities, DEP's Division of Fish and Wildlife personnel for Wildlife Management Areas, DEP's Water Compliance and Enforcement program, academia, Water Suppliers with surface water supplies, USGS, Rutgers Cooperative Extension, 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 the report relates to a drinking water source, the DEP DWSG will be contacted. See section 4.E. for communication actions.

Upon initial reporting of a suspected HAB, one or more of the following field screenings (See Section B below) will be performed by a qualified organization 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 5. Quick Reporting Guide

You can help!

If you observe what you think might be a HAB in a pond, lake, or stream, a suspected Harmful Algal Bloom report, can be submitted by smartphone or PC using the NJDEP HAB Interactive Map Reporting and Communication System. The HAB System will be used to gather initial information such as: location coordinates, photos, known recreational activities, and extent of the waterbody. This information will be used to inform DEP to initiate appropriate response actions. Once the DEP completes the investigation of the suspected HAB, results and any recommendations for public notices or advisories will be communicated through the HAB System. All information and HAB data will be accessible by clicking the location on the interactive map in the HAB System. If a smart phone or computer is not available, reports may also be submitted to the DEP Hotline at 1-877-WARNDEP (927-6337) - If reporting by phone, please note the exact location of the suspected HAB along with any details (e.g., date/time, bloom appearance and color, and if known, whether a swimming beach is nearby or whether the waterbody is a drinking water source like a reservoir).

B. Screening

Upon receiving a report of a suspected HAB, several screening procedures may be performed to inform continued response and confirmation actions.

i. Cyanobacteria Presence and Field Measurements

The presence of phycocyanin pigment (unique to cyanobacteria) can be determined using a handheld field fluorometer (phycocyanin meter). If a phycocyanin meter is not available, a sample may be collected for laboratory analyses. See Appendix B for the sample collection procedure for HABs. If using a non-DEP lab, assure samples are collected in amber glass bottles or amber plastic bottles made of polyethylene terephthalate glycol (PETG) or High-Density Polyethylene (HDPE), refrigerated, and analyzed within 24 hours. Exact sample size, collection materials, holding times, and preservation should be confirmed with the laboratory. The laboratory will provide all collection procedures and preservation to assure compliance with the minimum requirements of the analytical method.

ii. Visual Assessment

A visual assessment is an important part of the NJDEP HAB System. When public reports are received, usually the same or next day, the System requests information on size, extent, and visual information using example photos available in the System. Many times, a determination can be made simply based on a supplied photo. When samplers visit the waterbody, additional visual information and measurements are input into the system.

iii. Remote Sensing – Satellite Imagery, Aircraft Flight Reconnaissance and Unmanned Aerial Vehicles (UAVs)

While discrete laboratory analyses (cell identification and enumeration, and toxin analyses) serve as the definitive determination of whether results exceed NJ Health Advisory Guidance levels, remote

sensing data provides useful screening information on the spatial extent and relative cell density a bloom. Remote sensing is also a valuable tool to assess HAB trends (i.e., whether the HAB is increasing or dissipating).

Satellite imagery. Satellite imagery, such as the USEPA's Cyanobacteria Assessment Network Application (CyAN app) https://cfpub.epa.gov/si/si_public_record_Report.cfm?Lab=NERL&dirEntryId=346902. The CyAN app provides weekly satellite data to identify the concentration, location, and time series of cyanobacterial blooms in fresh and coastal waters of the United States. Monitoring this application may be used to inform decisions on staff deployment for other response actions such as field screening and sampling. Due to resolution limitations, satellite imagery is limited to the approximately seven largest lakes in the State (Wanaque Reservoir, Union Lake, Greenwood Lake, Boonton Reservoir, Lake Hopatcong, Lake Tappan, Round Valley Reservoir).

Aircraft Flight Reconnaissance.
The DEP has developed aircraft remote sensing capabilities for general cyanobacteria detection and tracking. A hyper-spectral sensor is used to detect wavelengths of light specific to the cyanobacteria pigment phycocyanin in a waterbody. This advanced monitoring method provides immediate feedback on the presence and relative cyanobacteria cell counts and can serve as a screening method to target waters for sample collection.

Unmanned Aerial Vehicles (UAVs)
DEP is also working on the development and use of UAVs for HAB screening through photography and remote sensing for phycocyanin. UAV surveillance can be used for smaller lakes than the satellite remote sensing.

iv. Continuous Data Monitoring Program

Continuous monitors may be deployed at waterbodies with recurring HABs or having recreational, drinking water, or ecological significance. Phycocyanin, as well as other water quality measurements, are monitored for the status of an existing HAB or for conditions that may predict the onset of a HAB (e.g. changes in pH or dissolved oxygen). Data from these continuous monitors will inform the deployment of staff for on-site measurements and sampling. Continuous monitoring data can be found here: http://njdep.rutgers.edu/continuous/

If cyanobacteria cell density is estimated to be above NJ Health Advisory Guidance levels using any of these screening methods, cell identification, enumeration and toxins will be analyzed per below.

v. Toxin Presence

A microcystins test strip reading is considered a semi-quantitative analysis and 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. Microcystins test strip results will be interpreted, per the manufacturer's instructions (Appendix C) in the following manner:

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 laboratory analysis may be performed for other toxins.

C. Confirmation Laboratory Analysis

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

Microcystins
Microcystins are a group of at least more than 200 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 *Dolichospermum (previously Anabaena), Fischerella, Gloeotrichia, Nodularia, Nostoc, Oscillatoria, members of Microcystis, and Planktothrix*.

Cylindrospermopsin
Cylindrospermopsin is usually produced by *Raphidiopsis (previously Cylindrospermopsis), raciborskii (C. raciborskii), Aphanizomenon flos-aquae, Aphanizomenon gracile, Aphanizomenon ovalisporum, Umezakia natans, Dolichospermum (previously Anabaena) bergii, Dolichospermum lapponica, Dolichospermum planctonica, Lyngbya wollei, Raphidiopsis curvata, and Raphidiopsis mediterranea.* 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 include 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 *Chrysosporum (Aphanizomenon) ovalisporum, Cuspidothrix, Raphidiopsis (previously Cylindrospermopsis), Cylindrospermum, Dolichospermum, Microcystis, Oscillatoria, Planktothrix, Phormidium, Dolichospermum (previously Anabaena) flos-aquae, A. lemmermannii, Raphidiopsis mediterranea (strain of Raphidiopsis raciborskii), Tychonema and Woronichinia.* (USEPA's HABs website: (https://www.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-water)

i. Toxin Analysis Methods

Samples analyzed by DWMS/BFBM laboratory will use a microtiter plate Enzyme-Linked Immuno-Sorbent Assay (ELISA), EPA method 546, using an automated plate reader (Figure 6) and ABRAXIS kits (Sample Collection Reference Guide Methods in Appendix B and C respectively). The DEP Office of Quality Assurance, Laboratory Certification Program offers certification for this method. This method was utilized by the USEPA as part of the National Lakes Assessment (NLA). Quality Assurance/ Quality Control (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).

Analysis levels (note levels are significantly below NJ Health Advisory Guidelines)

- Microcystins (> 80 variants)
  - Method – ELISA (EPA Method 546)
  - Detection limit = 0.10 µg/L
  - Reporting level = 0.15 µg/L
- Cylindrospermopsin
  - Method - ELISA.
  - Detection limit = 0.04 µg/L
  - Reporting level = 0.05 µg/L
- Anatoxin-a
  - Method – ELISA
  - Detection limit = 0.10 µg/L
  - Reporting level = 0.15 µg/L

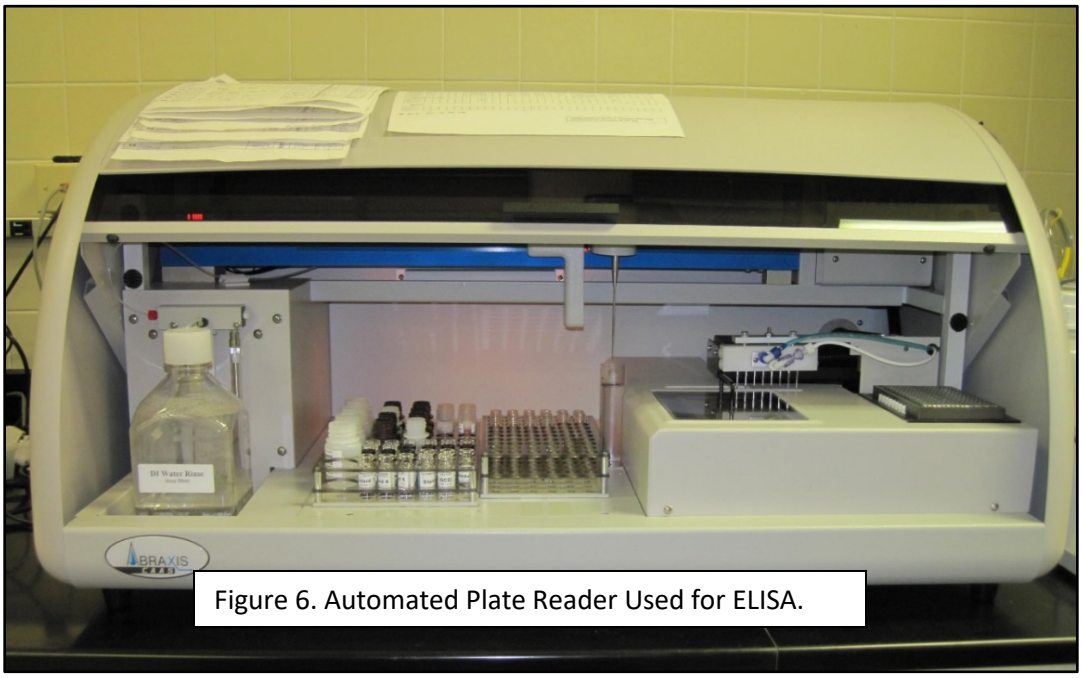


Figure 6. Automated Plate Reader Used for ELISA.

For detection of cyanotoxins in drinking water, EPA developed Method 544, a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for six 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. Additional information regarding UCMR4, the applicable water systems involved, and the timeframe and frequency of sampling can be found here: https://www.epa.gov/dwucmr/fourth-unregulated-contaminant-monitoring-rule.

ii. Chlorophyll 'a' and cell counts

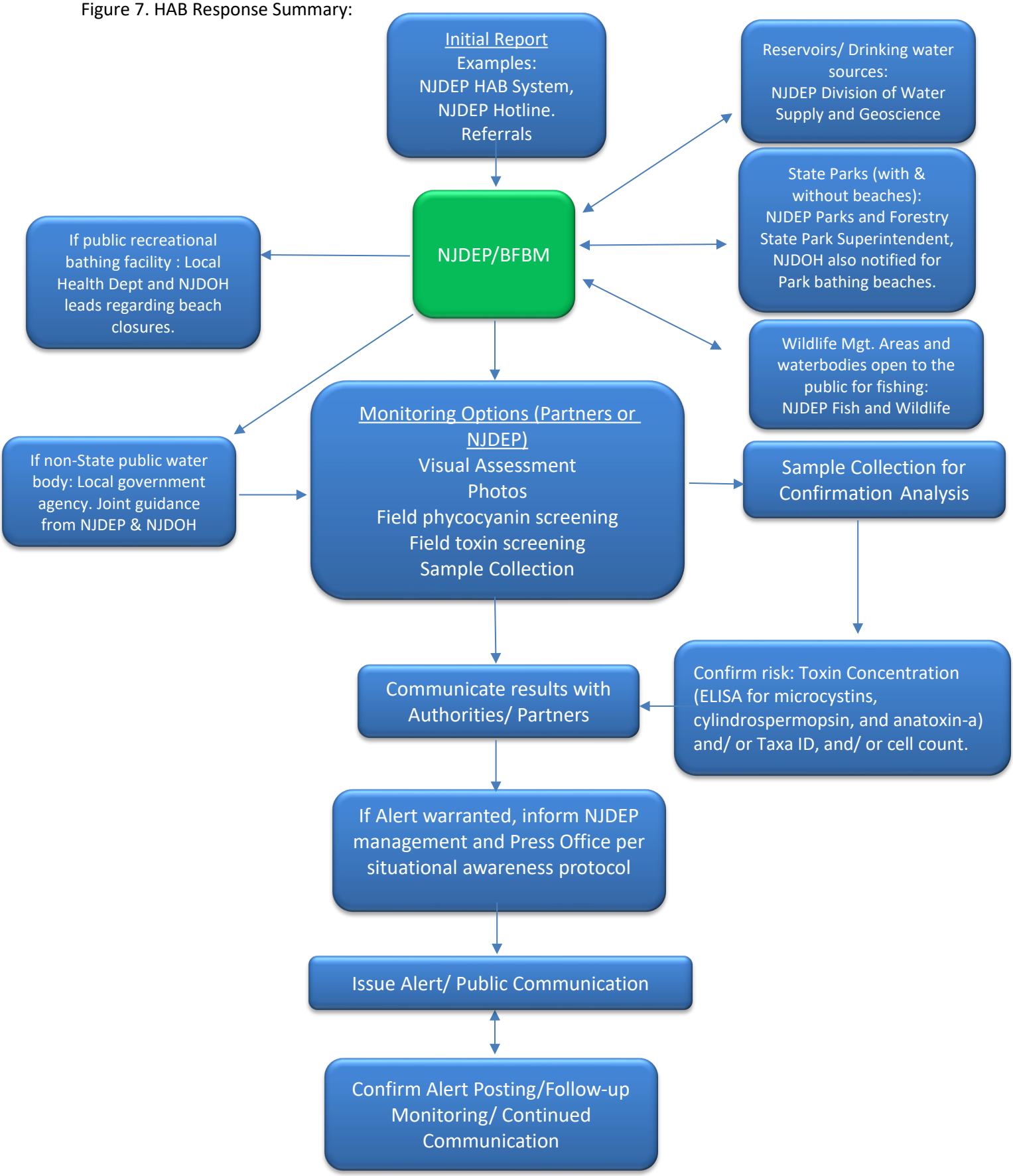
Algal concentrations in the water column are measured through Chlorophyll 'a' analysis. Chlorophyll "a" is contained in both green algae and cyanobacteria, both of which may be present in a bloom community at varying ratios. As a conservative estimate of possible health risk, it is assumed that higher 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 as an additional screening method or measure of relative abundance. WHO guidance for Chlorophyll 'a' and cell counts for moderate risk are Chlorophyll 'a' > 10 µg/l and cell counts > 20,000 – 100,000 cells/ml (Appendix D). WHO report is available at: http://www.who.int/water_sanitation_health/publications/srwe1/en/.

E. Response/ Actions

Depending on the waterbody and its use, a variety of actions may be taken by DWMS/BFBM to communicate risk to the proper authority and the public. (Figure 7 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 bathing beach, the specific State Park Superintendent and DOH will be notified.
- If reported at a Public Recreational Bathing facility (PRB), other than a State Park, the appropriate local health department and DOH will be notified. DOH will convey recommended actions to local health departments.
- If reported at a State Park recreational water that is not a bathing beach, 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 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.
- DEP will make every effort to respond to reported suspected HABs as soon as possible. In the event that resources are limited, the response actions will be prioritized based on potential risk to public health.
    1. Drinking water sources.
    2. Bathing beaches (PRBs).
    3. Recreational waters without bathing beaches.
    4. Waterbodies with a protective alert already in place.
    5. Waterbodies not covered in the above.

Figure 7. HAB Response Summary:



F. Communication/ Continued Monitoring

A tiered approach will be used for notices and advisories based on analysis results from response and continued monitoring. If levels are above NJ Health Advisory Guidance for toxins and/or cell concentrations, it is recommended that advisories be posted or PRB closures implemented (See Section 5). Situational awareness in accordance with established internal DEP protocols will be initiated. After initial HAB confirmation and actions, subsequent monitoring may be necessary until the risk level subsides or the HAB dissipates. 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 waterbody 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 responsible authorities.

After initial response and issuing of an advisory, it is the responsibility of the resource's authority (e.g., Division of Fish and Wildlife, local health department) to communicate any substantial changes in status such as increased discoloration or dissipation of the HAB to DWMS throughout the HAB event, until the advisory is lifted. An agreed upon surveillance frequency which will consider recreational use, HAB extent, and other factors will be employed. Screening or visual observations which indicate a potential increase in cell counts or toxin production may result in additional DWMS/BFBM response and monitoring.

5. CYANOBACTERIAL HARMFUL ALGAL BLOOM ADVISORIES

The tiered Alert levels are based on the recommended NJ Health Advisory Guidance Levels for Recreational Exposure. The tiered Alerts are intended to be protective for the exposures most likely to occur from recreational activities. Two categories of recreational activity are defined per the USEPA (2004) Water Quality Standards for Coastal and Great Lakes Recreation Waters. Proposed Rule as follows: "Primary contact recreation is typically defined by States and Territories to encompass activities that could be expected to result in the ingestion of, or immersion in, water, such as swimming, water skiing, surfing, kayaking, or any other activity where immersion in the water is likely." Secondary contact recreation consists of the following activities that may result in incidental contact with water, but not full body immersion in, nor ingestion of, water: wading, fishing, hunting, power boating, canoeing, sailing (ORSANCO, 2018).,

When posting advisories, it is recommended to err on the side of caution to avoid unnecessary risk to the public. These advisories may be modified on a site-specific basis as appropriate to reflect the nature and extent of a specific HAB occurrence.

DEP has developed Alert Levels (Watch, Alert, Advisory, Warning and Danger) based on cyanobacterial cell concentrations and cyanotoxin levels in a bloom that can be used to provide tiered advice for recreational exposure to HABs and their toxins. These tiered Alert Levels are based on DSR's evaluation of potential health effects at elevated microcystin concentrations, as well as Warning and Danger (or similar) guidelines from WHO and other states. More detail on the basis for the tiered Alert levels is found in Appendix E.

Watch

A Watch should be used if a HAB is strongly suspected based on visual, photographic or other screening measures such as phycocyanin measurements, or if laboratory analysis results confirm that cyanobacteria are present, and cell concentrations are >20,000 cells/ml and < 80,000 cells/ml and toxins are below Health Advisory Guidelines. While there is no recommendation suggesting the need to limit recreational activities, caution should be used and contact with visible blooms should be avoided. Precautionary beach closures may be put into place by a local health department/authority or a PRB owner/operator if visual or other clear evidence of a HAB is present until confirmation analysis is performed. Additionally, a cell concentration >40,000 cells/ml and < 80,000 cells/ml at PRBs initiates an Alert for additional monitoring as per below:

Alert Tier for Public Recreational Bathing Facilities (PRB)

An Alert applies to PRBs only. An Alert should be used if laboratory analysis results confirm that cyanobacteria are present, and the cell concentration is > 40,000 cells/ml and < 80,000 cells/ml, and toxins are below Health Advisory Guidelines. An Alert initiates actions by the DEP or partners to monitor the waterbody more closely for changes in the HABs appearance. Such changes may indicate an increase in cell concentrations or toxin production warranting the collection of additional samples. The Watch advice remains in effect. No limits in recreational activities are suggested; however, caution should be used and contact with visible blooms

should be avoided. Precautionary beach closures may be put into place by the local health department or authority or the PRB facility owners/operators if visual or other clear evidence of a HAB is present.

Advisory

An Advisory should be used if a HAB is confirmed through laboratory analysis within the health advisory guidance levels range for cell concentration of > 80,000 cells/ml or above any health advisory guidance level for measured toxins.

Public Recreational Bathing Beaches (PRBs)

Upon confirmation analysis*, PRBs will be closed under the authority of DOH regulation, New Jersey State Sanitary Code Chapter IX Public Recreational Bathing N.J.A.C. 8:26.

DOH will communicate advisory recommendations to local health departments and confirm PRB Closures have been carried out appropriately.

*If there is compelling evidence at a PRB (e.g, field measurements using a fluorometer), the local authority may close the PRB until confirmation analysis is performed.

Areas with no PRBs

An Advisory may be posted at public access points in waterbodies, or sections of waterbodies, where a PRB is not present, but other recreation or use may occur. At these areas, primary contact recreation is not advised. While there is no recommendation against secondary recreational activities, caution should be used and contact with visible blooms should be avoided

Warning*

A Warning should be issued if a HAB is confirmed through laboratory analysis with microcystins toxin levels of >20 µg/L and <2000 µg/L. PRBs will be closed and Warning signs posted as above. At these areas, primary contact recreation is not advised. Secondary contact recreation may not be recommended if additional evidence (e.g., animal or human adverse health effects reports) exists.

Danger*

A Danger posting will be considered if microcystins toxin levels are > 2000 µg/l and there is a significant increased risk to public health. A Danger notification will prohibit all primary and secondary contact recreation activity for the waterbody. A waterbody closure, or partial closure, may be considered after evaluating all aspects of the HAB event, including but not limited to recreational uses, size and extent of bloom and monitoring data.

***The intent of these tiers is to advise against secondary recreation when a HAB poses an imminent threat to public health and safety, or if the HABs results in the confirmed injury/death of wildlife, pets or livestock. Therefore, other evidence, such as reported health effects, may be used to recommend the posting of these tiers.

Recommended Alert Levels:

Table 2. Summary of Alert Levels, Criteria, and Recommended Recreational Activities.

HAB ALERT LEVEL	CRITERIA	RECOMMENDATIONS
NONE	HAB report investigated and no HAB found	None
WATCH *Suspected or confirmed HAB with potential for allergenic and irritative health effects*	Suspected HAB based on visual assessment or screening test **OR** Lab confirmed cell counts between 20k – 40k cells/mL **AND** No known toxins above public health thresholds	Public Bathing Beaches Open (dependent upon local health authority evaluation and assessment) Waterbody Accessible: • Use caution during primary contact (e.g. swimming) and secondary (e.g. non-contact boating) recreational activities Do not ingest water (people/pets/livestock) Do not consume fish
ALERT *Confirmed HAB that requires greater observation due to increasing potential for toxin production* **PUBLIC BATHING BEACHES INCREASE MONITORING**	Lab confirmed cell counts between 40k – 80k cells/mL **AND** No known toxins above public health threshold	**WATCH remains in effect.** **Public Bathing Beaches Open (dependent upon local health authority evaluation and assessment) and should observe and report changing bloom conditions** Waterbody Accessible: • Use caution during primary contact (e.g. swimming) and secondary (e.g. non-contact boating) recreational activities Do not ingest water (people/pets/livestock) Do not consume fish
ADVISORY *Confirmed HAB with **moderate risk of adverse health effects** and increased potential for toxins above public health thresholds*	Lab testing for toxins exceeds public health thresholds **OR** Lab confirmed cell counts above 80K cells/mL **OR** Field measurement evidence indicating HAB present and above guidance thresholds (e.g. phycocyanin readings)	Public Bathing Beaches Closed Waterbody Remains Accessible: • Avoid primary contact recreation (e.g. swimming) • Use caution for secondary contact recreation (e.g. boating without water contact) Do not ingest water (people/pets/livestock) Do not consume fish
WARNING *Confirmed HAB with **high risk of adverse health effects** due to high toxin levels*	Toxin (microcystin) 20 - 2000 µg/l **AND/OR** Additional evidence, including, expanding bloom, increasing toxin levels (i.e. duration, spatial extent or negative human or animal health impacts) indicates that additional recommendations are warranted	Public Bathing Beaches Closed Waterbody Remains Accessible: • Avoid primary contact recreation (e.g. swimming) • May recommend against secondary contact recreation (e.g. boating without water contact) with additional evidence Do not ingest water (people/pets/livestock) Do not consume fish
DANGER *Confirmed HAB with **very high risk of adverse health effects** due to very high toxin levels*	Toxin (microcystin) > 2000 µg/l **AND/OR** Additional evidence, including, expanding bloom, increasing toxin levels (i.e. duration, spatial extent or negative human or animal health impacts) indicates that additional recommendations are warranted	Closure of Public Bathing Beaches Possible closure of all or portions of waterbody and possible restrictions access to shoreline. Avoid primary contact recreation (e.g. swimming) May recommend against secondary contact recreation with additional evidence Do not ingest water (people/pets/livestock) Do not consume fish

WHO (2003) states that a ***relatively low probability of adverse health effects from cyanobacteria*** is due to the irritative or allergenic effects of cyanobacterial components and exists at a cyanobacterial cell concentration of 20,000 cyanobacterial cells/ml; these effects are not due to cyanotoxin toxicity. In studies of individuals with recreational exposure to cyanobacterial blooms, health outcomes were related to cyanobacterial density and duration of exposure, and less than 30% of individuals were affected at a cell concentration of 20,000 cells/ml. WHO (2003) further states that a **moderate probability of adverse health effects** occurs at higher concentrations of cyanobacterial cells, and the probability of irritative symptoms is elevated. Additionally, cyanotoxins may reach concentrations with potential health impacts at higher cell concentrations. (WHO, 2003).

Public Bathing Beaches will be closed under the authority of NJDOH regulation, New Jersey State Sanitary Code Chapter IX Public Recreational Bathing N.J.A.C. 8:26. If there is compelling evidence at a PRB from visual surveillance or through field measurements (e.g., phycocyanin meter), the local health department/authority has the authority to close the PRB until confirmation analysis is performed.

NOTE: A printable version of HAB signs can be found on the web page below:
http://www.state.nj.us/dep/wms/bfbm/advlanguage.html

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

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

Watch/Alert

- Continue field surveillance for substantial changes in bloom conditions. If changes occur, perform laboratory analysis to confirm that levels remain below thresholds. Analysis frequency to be determined on a case-by-case basis.
  Watch should remain in effect until HAB has visually dissipated and laboratory analysis confirms that levels remain below thresholds, or until analysis confirms that the HAB has worsened, and exceeds the Advisory Level or higher Alert Level.

Advisory/ Beach Closure

- **Public recreational bathing facility**
  - If HAB is present with cell count or toxin levels quantified at or above the health advisory guidance levels, the PRB closure should not be lifted until:
    - With no phycocyanin field measurements - two (2) subsequent lab analyses are below cell count and toxin thresholds, or
    - If phycocyanin measurements show levels are below thresholds for 5 consecutive days, then only one laboratory analysis with cell count and toxin results below thresholds is necessary.
  - When advisory is lifted, and/ or PRB is re-opened, the DOH recommends continued frequent surveillance of the waterbody and documentation of findings (visual and/ or phycocyanin). Follow-up laboratory analysis is required when bloom appearance changes or phycocyanin measurements increase.

- If a HAB re-occurs (visual and/ or phycocyanin), then automatic closure of the PRB until thorough testing is conducted and no cell count or toxin levels are detected above thresholds.
- Any re-opening of PRBs will be communicated by DOH to the local health department. If at any time after re-opening a HAB has re-occurred based on visual observations or phycocyanin measurements, the PRB should be closed immediately and sampling/ analysis initiated.

- **Areas with no PRBs**

- If HAB is present with cell counts or toxin levels quantified at or above the health advisory guidance levels, the Advisory should not be lifted until one subsequent analysis is below thresholds.
- When Advisory is lifted, continue surveillance of the waterbody using the suggested screening procedures in Section 4.B, and document findings. If a HAB re-occurs, then follow-up laboratory analysis is required.

Warning and Danger

Actions performed as above Advisory tier. However, additional monitoring and analysis may be necessary depending on the severity of the HAB and its impact on the waterbody use, and the frequency of such additional monitoring will be determined on a case by case basis. Such analyses may indicate the downgrading of advice to lower level Alert tiers, as well.

6. RESEARCH STRATEGY

DEP's DSR and DWMS/BFBM co-chair the HAB Research Committee which provides technical consultation regarding HAB bloom response, implements portions of the Science Agenda component of the Governor's Harmful Algal Blooms (HABs) Initiative, and conducts literature-based evaluations and applied research on the following topics:

- New developments in HAB screening, monitoring and laboratory analysis
- Downstream fate and transport of cyanobacteria and toxins
- Factors that contribute to toxin production
- Risks of consumption of fish from waters where HABs are present, including commonly caught game fish.

Literature research will include keeping abreast of HAB monitoring and response strategies established by other states, current USEPA guidance, and studies reported by United States Geological Survey, academic researchers, and others.

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

- Technology
    - Investigation of the application of new analyses, monitoring equipment and surveillance equipment, such as:
        - Use of satellite imagery, monitoring aerial unmanned vehicles, and other aircraft-based sensor technology to monitor cyanobacterial blooms.
        - Flow cytometer and Luminex Assays as potential monitoring methods.
        - Molecular PCR and qPCR techniques for identification and quantification of cyanobacteria and toxin production potential.
- Pilot Studies
    - Coordination with academia and other local agencies to develop enhanced monitoring and detection techniques.
- Predictive Tools/Prevention
    - Use of water quality data, bathymetry, weather/ climate, land use and other information to predict possible HAB events and/or prevent such events through lake management.
- Treatment
    - In consultation with the HAB prevention and mitigation Expert Team developed for the Governor's Harmful Algal Blooms (HABs) Initiative, build on existing efforts to develop a database of treatment technologies.
    - Evaluate 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, to other State and local agencies, as well as to the public. Communication mechanisms which continue to be pursued include, but are not limited to:

- Implementation of "improve communication" component of the Governor's Harmful Algal Blooms (HABs) Initiative.
    - Development of a new and improved overall HAB website, including updated scientific information.
    - Development of a new interactive HAB mapping and communication system.
- Continue development of new and revision of existing fact sheets and other outreach material (e.g., general information posters and post cards) for intra-Departmental, other government agency, partners and public use.
- Continue maintaining and enhancing both overall DEP HAB website (https://www.nj.gov/dep/hab/) as well as BFBM CyanoHAB website (https://www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html)
- Continue making all outreach material available for download at: http://www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html. Outreach material will include, but is not limited to:
- Continue to develop DEP HAB Fact Sheets/ Developed and update as new information becomes available
    - Cyanobacterial Harmful Algal Blooms (HABs)
    - Cyanobacteria Harmful Algal Blooms (HABs) and Cyanotoxins: Recreational Exposure and Health Effects
    - Harmful Algal Blooms and Pets
- Continue developing and/or refining physical signage to be used in response to suspected or confirmed HABs.
- Continue communication/ coordination on HABs, and development of surveillance and monitoring partnerships with the members of 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.
- Continue communication/coordination with county and local health departments through avenues such as the County Environmental Health Act (CEHA) program and the Cooperative Coastal Monitoring Program (CCMP).
- Continue training and information exchange for DEP programs, partners and the public, such as in-person training, webinars, videos and web- based training.
- Development and/or use of existing Smart phone apps, for identifying, reporting, and communicating potential HAB concerns.
- Continue working with State Park Service and Division of Fish and Wildlife to provide and enhance, where necessary, information that would be accessible at New Jersey State Parks and Wildlife Management Areas. Items include physical signage, informational material, increased information on individual park and wildlife management area websites, etc.
- Explore partnering with other state agencies in the region to adapt existing communication efforts for New Jersey.
- Explore various additional platforms for communicating HABs information, including social media and listservs.

- Investigate use of the 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. ORSANCO. Ohio River Valley Residents' River-Based Recreation and Consumption of Freshwater Fish. 2018. http://www.orsanco.org/wp-content/uploads/2018/07/Ohio-River-Valley-Residents-River-Based-Recreation-and-Consumption-of-Fresh-Water-Fish.pdf
3. USEPA. Developing a Cyanobacteria Monitoring & Bloom Watch Program | EPA & Minnesota Sea Grant. November 9, 2016.
4. USEPA. 40 CFR Part 131 [OW–2004–0010; FRL–7785–6] RIN 2040–AE63 Water Quality Standards for Coastal and Great Lakes Recreation Waters.
5. USEPA's HABs website: https://www.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-water
6. USEPA. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. EPA 822-P-19-001. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC. 2019.
7. 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.
8. USEPA. Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water. Office of Water (4606M). EPA 815-R-15-010. June 2015.
9. 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.
10. USGS. Loftin, K. A., Ziegler, A. C., and Meyer, M. T. 2008. Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs. U.S. Department of the Interior, U.S. Geological Survey
11. World Health Organization. Guidelines for Safe Recreational Water Environments. 2003. 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 and Workgroup Agency Contact Information

New Jersey Harmful Algal Bloom (HAB) Workgroup

DEP DWMS
Leslie McGeorge
Victor Poretti
Tom Miller
Dean Bryson
Alena Baldwin-Brown
Johannus Franken
Mike Kusmiesz
Bob Schuster
Ismail Sukkar
Rachel White
Aynan Zaman
Bruce Friedman
Tracy Fay
Chris Kunz

DEP DSR
Robert Newby
Gloria Post
Nick Procopio

DEP WRM
Jim Lunski
Monique Girona

DEP Water Supply and Geoscience
Matthew Wilson
Kelley Meccia
Christian Haviland
Chelsea Brook

DEP State Park Service
Blanca Chevrestt, Northern Region
Jonathan Luk, Central Region
Lorraine McCay, Southern Region
Jenny Felton, Spruce Run
Josh Osowski, Regional Superintendent
Northern Region Office

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

DEP Office of Quality Assurance
Melissa Hornsby

DOH Division of Epidemiology, Environmental and Occupational Health/Consumer, Environmental and Occupational Health Service (CEOHS)
Loel Muetter
Danielle Clemons
Gary Centifonti

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
Manoel Tamassia
Sebastian Reist

Workgroup Agency Contact Information

DEP

DEP HAB Reporting and Communication System:
https://survey123.arcgis.com/share/6335130701574e688500f7c5556fc2b3.

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

https://www.state.nj.us/dep/hab/

DEP Division of Water Monitoring and Standards
http://www.nj.gov/dep/wms/
njcyanohabs@dep.nj.gov

DEP Bureau of Freshwater and Biological Monitoring (BFBM)
609 -292-0427
http://www.state.nj.us/dep/wms/bfbm/CyanoHABHome.html

DEP Division of Science and Research
609-940-4080
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/oqa.html

New Jersey Department of Health (DOH)

AFTER HOURS EMERGENCY CONTACT
609-392-2020

NJDOH Public Health and Food Protection Program (PHFPP):
http://www.nj.gov/health/ceohs/sanitation-safety/environmental/
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/

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 B – HAB Sample Collection Method

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

HAB Field Collection Procedure For DEP BFBM Laboratory Analyses

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, often most frequently in the summer or fall. Algae-like bacteria which occur primarily in freshwater, or cyanobacteria 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 of cyanobacteria is Microcystis which can produce a common group of toxins called microcystins, as well other toxins. Microcystins may cause adverse health effects to humans and animals, if ingested, if contacted by skin or mucous membranes, or if inhaled. Other types of cyanotoxins, include anatoxin and cylindrospermopsin.

The procedure for field sample collection provided below is for analyses at DEP's BFBM HAB laboratory. If collecting water samples for analyses at another laboratory, that facility should be contacted for their specific field sample collection procedures.

SAMPLING PROCEDURES for ANALYSIS AT DEP's BFBM HAB LABORATORY

Equipment and Supplies

- Protective gloves
- 500 ml bottles
- BFBM labels
- Cooler with ice.

Notifications

- A Harmful Algal Bloom report, can be submitted by smartphone or PC using the NJDEP HAB Reporting and Communication System. The HAB Reporting and Communication System will be used to gather initial information such as: location coordinates, photos, known activities, and extent over the waterbody. This information will be used to inform DEP to initiate appropriate response actions. Once the DEP completes the investigation of the suspected HAB, results and

recommendations for public notices or advisories will be communicated through the HAB System. All information and data will be accessible to the public by clicking the location on the interactive map in the HAB System. If a smart phone or computer are not available, reports may also be submitted to the DEP Hotline at 1-877-WARNDEP (927-6337).

- Upon receipt of report, BFBM will contact partner to coordinate sampling and to assure the correct measurements are recorded and necessary sampling supplies are in hand.
- 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. Brown plastic bottles made of polyethylene terephthalate glycol (PETG) or High Density Polyethylene (HDPE), wrapped in foil may be used as an alternative to glass.
- Samples should be collected just below the surface so mouth of bottle is immersed approximately 3-6 inches. (make sure algae is represented in sample)
- Fill out label with permanent marker and place on sample bottle.
- 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

APPENDIX C - Cyanotoxin Analysis Methods and Specifications

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 (µg/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 µg/L (ppb)
-For all other age groups, 1.6 µg/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.

For ordering or technical assistance contact:

Abraxis, Inc.
124 Railroad Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

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-dosing, 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.

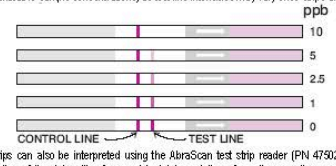
1. Using a new graduated disposable pipette for each sample, draw the sample to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of sample to the lysis vial.
2. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes, to begin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

Control Line	*Test Line*	*Interpretation*
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, 4849-4856.
(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 lead 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.

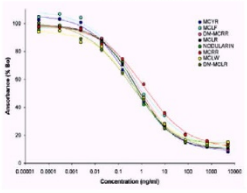
To protect consumers from adverse health effects caused by these toxins, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water.

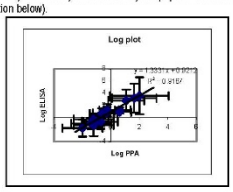
Performance Data

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, 4849-4856.
(2) Worldwide Patenting PCT WO 01/18059 A2.
(3) U.S. Patent Number 6,967,240.

For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

ETV EPA

R3113150H

Microcystins-ADDA ELISA (Microtiter Plate)

ABRAXIS

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 µL)
2. Multi-channel pipette (50-300 µL), stepper pipette (50-300 µL), 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 µm 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.

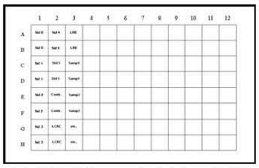
E. Test Preparation

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.

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 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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/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 Microcystins concentration on the horizontal logarithmic (x) axis on graph paper. %B/B$_0$ 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 lead 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 Cylindrospermosin 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

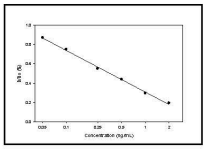
Test sensitivity: The detection limit for this assay is 0.040 ppb (µg/L).

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 (%)
	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.**

For ordering or technical assistance contact:

Abraxis LLC
124 Railroad Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

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. Cylindrospermopsin-HRP Conjugate Solution
6. Antibody Solution (rabbit anti-Cylindrospermopsin)
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 µL)
2. Multi-channel pipette (50-300 µL), stepper pipette (50-300 µL), 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.

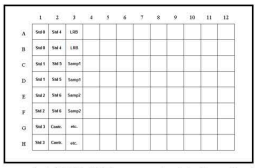
E. Test Preparation

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.

Sd0-Sd6: Standards
Contr.: Control (QCS)
LRB: Laboratory Reagent Blank
Samp1, Samp2, etc.: Samples



G. Assay Procedure

1. Add **50 µL 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 µL of the enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50 µL 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 µL 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 µL 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 µL 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 Cylindrospermopsin 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 Cylindrospermopsin 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 Cylindrospermopsin than standard 1 (0.05 ppb) should be reported as containing < 0.05 ppb of Cylindrospermopsin. 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 Cylindrospermopsin greater than that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of Cylindrospermopsin less than that calibrator.

I. References

(1) Cylindrospermopsin, 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/B_0) is approximately 0.1 ppb (µg/L). The middle of the test (50% B/B_0) 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%

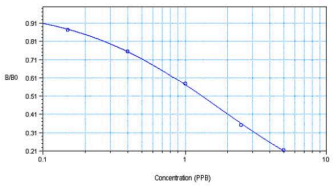
Recoveries:

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 (UVEG).

For ordering or technical assistance contact: Abraxis, Inc.
124 Railroad Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

R053116

Anatoxin-a ELISA Microtiter Plate

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

ABRAXIS

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 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-300 µL)
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

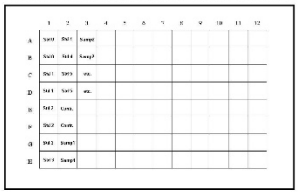
Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods across the entire microtiter 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.

Std 0-Std5: Standards
Contr.: Control
Samp1, Samp2, etc: Samples



F. Assay Procedure

1. Add **50 µL 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 µL of the reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50 µL 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 µL 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 µL 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 µL 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 D
World Health Organization (WHO) and USEPA Recreational HAB Guidance

The World Health Organization (WHO) established guidelines for cyanobacteria in their *Guidelines for Safe Recreational Water Environments. 2003*. **http://www.who.int/water_sanitation_health/publications/srwe1/en/**.
For recreational waters, the World Health Organization (WHO) concluded 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 was necessary to differentiate between the chiefly irritative symptoms caused by cyanobacterial substances and the more severe health effects due to exposure to high concentrations of known cyanotoxins, particularly microcystins. (WHO, 2003). WHO provided a series of recreational guidance/action levels for cyanobacteria, microcystins and chlorophyll a.

In 2019, USEPA released two final recreational cyanotoxin values in *Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin*(USEPA, 2019). Although USEPA did not recommend specific recreational numeric criteria or swimming advisory values for cyanobacterial cell counts and/or biomass, the Agency indicated that, together with microscopic identification, these measures can be informative in making public health decisions and/or in prompting toxin analysis. The Recreational Criteria /Swimming Advisory document also included the information that it has been established that some sensitive individuals have adverse allergenic/irritative responses from exposure to cyanobacterial cells at concentrations as low as 5,000 cells/ml (USEPA, 2019).

The USEPA 2019 HAB Recreational Criteria/Swimming Advisory document summarizes the 2003 WHO HAB guidance in the table below:

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

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

APPENDIX E
Basis for Health Advisory Guidelines
1. Basis for NJDEP Reference Doses for Cyanotoxins
2. Background Information on Microcystin "Warning" and "Danger" Threshold Values

Basis for NJDEP Reference Doses for Cyanotoxins

Gloria B. Post, Ph.D., DABT
Division of Science and Research
NJDEP
April 14, 2020

Summary

Recreational advisories for cyanotoxins are based on short-term Reference Doses. The scientific basis of the short-term Reference Doses for microcystin-LR, cylindrospermopsin and anatoxin-a were developed by the New Jersey Department of Environmental Protection (NJDEP) Division of Science and Research (DSR) in 2017 (NJDEP, 2017). The basis for these Reference Doses was reviewed in 2020 to determine whether any new information is available that would indicate updates are needed. Relevant recently published peer-reviewed studies were identified through a literature search and reviewed by DSR. This document provides the results of this review for these three cyanotoxins, including a comparison of the NJDEP and USEPA Reference Doses for microcystin-LR and cylindrospermopsin; there is no USEPA Reference Dose for anatoxin-a. Fifteen relevant new studies were identified and reviewed for microcystin-LR, one relevant new study was identified and reviewed for cylindrospermopsin, and no relevant new studies were identified for anatoxin-a. The new studies provide additional support for the Reference Doses of 0.01 µg/kg/day for microcystin, 0.03 µg/kg/day for cylindrospermopsin, and 0.1 µg/kg/day for anatoxin-a that were developed by DSR in 2017. As such, no updates to the Reference Doses are recommended at this time.

Introduction

New Jersey Department of Environmental Protection (NJDEP) Reference Doses used as the basis for recreational advisories for three cyanotoxins (microcystin-LR, cylindrospermopsin and anatoxin-a) were developed by the Division of Science and Research in 2017 (NJDEP, 2017) The basis of these Reference Doses was reviewed in 2020. The review included a literature search to identify relevant recently published peer-reviewed studies.

Recreational advisories for cyanotoxins are intended to be protective for children's swimming exposures during cyanobacteria harmful algal bloom (cyanoHAB) events, since children are the sensitive sub-population for swimming exposures. In New Jersey, cyanoHABs may persist for several months during the swimming season, and the recreational advisories are intended to protect for repeated daily exposures during the duration of a cyanoHAB event. Toxicity is considered through a short-term Reference Dose (µg/kg/day), which is the daily oral dose that is not expected to result in adverse health effects from short-term repeated exposures during a cyanoHAB event.

Process used to review basis of NJDEP (2017) Reference Doses

The PubMed database was searched 2015 through October 2019 for recent relevant studies that were not available when the NJDEP (2017) Reference Doses for microcystin-LR, cylindrospermopsin, and anatoxin-A were developed. These Reference Doses are intended to protect for repeated exposures to cyanotoxins during a cyanoHAB event. Relevant studies include repeated-dose oral (gavage or drinking water) studies with one of these cyanotoxins in mammalian species. Single-dose studies and studies in which dosing was via injection are not used as the primary basis for Reference Doses. Relevant recently published review articles were also considered, as discussed below.

Microcystin-LR Reference Dose

Basis of current NJDEP (2017) Reference Dose

The current NJDEP short-term Reference Dose (NJDEP, 2017) for microcystin-LR is 0.01 µg/kg/day. It is based on decreased weight gain and changes indicative of liver toxicity in mice dosed with microcystin-LR for 91 days (Fawell et al., 1994; 1999). The Lowest Observed Adverse Effect Level (LOAEL) in this study was 40 µg/kg/day, the lowest dose used, and a No Observed Adverse Effect Level (NOAEL) was not identified. Because the effects at 40 µg/kg/day were not severe, an uncertainty factor of 3, instead of the standard factor of 10, was used for LOAEL-to-NOAEL extrapolation.

As discussed in detail in NJDEP (2017), several other studies with durations applicable to development of a Reference Dose for recreational exposures to cyanotoxins during cyanoHAB events were reviewed, including short-term (>24 hours to 1 month) and subchronic (>1 month to 3 months) studies. These studies reported male reproductive, neurobehavioral and neurodevelopmental effects at doses (0.5 – 0.79 µg/kg/day) that are **50 to 80-fold lower** than 40 µg/kg/day, the LOAEL from Fawell et al. (1994; 1999). While these studies were not used as the primary basis for the Reference Dose because of issues related to their conduct and/or reporting, they suggested that **microcystin-LR causes toxicity at doses far below the dose** (40 µg/kg/day) used as the Point of Departure for the Reference Dose (NJDEP, 2017). As such, the NJDEP (2017) Reference Dose includes a database uncertainty factor of 10 to account for potentially more sensitive effects at lower doses.

Comparison with USEPA (2015a) Reference Dose

The USEPA (2015a) short-term Reference Dose for microcystin-LR is 0.05 µg/kg/day. It is based on a LOAEL of 50 µg/kg/day for liver toxicity (Heinze, 1999) that is very close to the LOAEL of 40 µg/kg/day for liver toxicity (Fawell et al., 1994/1999) used by NJDEP (2017).

The difference between the USEPA (2015a) and NJDEP (2017) Reference Doses is almost entirely due to the difference in the database uncertainty factor to account for data gaps and

potentially more sensitive effects. USEPA (2015a) also reviewed the studies mentioned above that showed effects at much lower doses, but used a partial database uncertainty factor of 3, while, for reasons discussed above, NJDEP (2017) used a full database uncertainty factor of 10. The other uncertainty factors used by USEPA are identical to those used by NJDEP (2017).

Additional recent toxicity studies

The PubMed literature search identified 15 peer-reviewed publications reporting on recent oral repeated-dose mammalian toxicity studies of microcystin-LR that were not considered by NJDEP (2017) or USEPA (2015a) in microcystin-LR Reference Dose development. In general, the newer studies do not have the methodological or reporting issues found in some of the older studies reporting effects at lower doses. These newer studies are summarized in Table 1.

The 15 publications included 6 studies in which the duration of exposure to microcystin-LR was 90 days or less. These studies are most relevant to development of short-term Reference Doses used as the basis for cyanotoxin recreational advisories. In all 6 of these studies, the LOAEL was lower than the LOAEL of 40 µg/kg/day used in the NJDEP (2017) Reference Dose, as summarized below. It should be noted that the doses in the drinking water studies are based on typical daily water consumption in mice of 0.2 ml/g/day[1] used to estimate the doses in several of the papers that were reviewed:

- He et al. (2017). Changes in metabolic profile and liver histology indicative of non-alcoholic fatty liver disease (NAFLD) occurred in mice after 90 days of exposure via gavage with a LOAEL of **20 µg/kg/day**. This was the lowest dose used, and a NOAEL was not identified.
- Lad et al. (2019). Biochemical and histological changes indicative of liver damage occurred in a strain of mice that is genetically modified to be a model for non-alcoholic fatty liver disease (NAFLD), after 28 days of gavage exposure with a LOAEL of **25 µg/kg/day**. This was the lowest dose used, and a NOAEL was not identified.
- Pan et al. (2018). Focal hyperplasia of the prostate occurred in mice after 90 days of drinking water exposure with a LOAEL of **2 µg/kg/day**. The NOAEL was 0.2 µg/kg/day. Effects were more severe after 180 days of exposure.
- Sedan et al. (2015). Lipid accumulation in the liver and decreased intraepithelial lymphocytes (immune system cells) in the small intestine occurred in mice after 30 days of gavage exposure with a LOAEL of **25 µg/kg/day**. This was the lowest dose used, and a NOAEL was not identified.
- Zhang et al. (2017). Effects on the reproductive system (decreased anogenital distance; decreased relative prostate weight, histopathological changes in the prostate, decreased

[1] The water consumption rate may have been lower than 0.2 ml/g/day based on estimates for mice provided by EFSA (2011) of 0.18 ml/g/day in subacute studies and 0.15 ml/g/day in subchronic study. If water consumption was lower than 0.2 ml/g/day, the doses (µg/kg/day) would also have been lower than those stated herein.

serum testosterone) occurred in 90-day old male offspring of female mice exposed through drinking water during pregnancy and lactation (12th day of gestation to 21st day after delivery). The LOAEL was **0.2 µg/kg/day**, the lowest dose used, with no NOAEL identified. Offspring were exposed prenatally and through breast milk, and potentially through direct access to drinking water at age 17-21 days.

- Zhou et al. (2020). An increase in the percentage of abnormal sperm tubules occurred in male mice after 90 days of drinking water exposure at a LOAEL of **20 µg/kg/day** and NOAEL of 2 µg/kg/day. With 180 days of exposure, the LOAEL was 2 µg/kg/day and the NOAEL was 0.2 µg/kg/day.

Nine additional studies with exposure durations longer than 90 days reported effects in mice at low doses including histological changes in the small intestine at 0.2 µg/kg/day (Cao et al., 2019), histopathological thyroid changes and decreased thyroid hormones at 2 µg/kg/day (Chen et al., 2019), histopathological changes in the lung at 1 µg/kg/day (Li et al., 2016), increased percent abnormal sperm at 1.5 µg/kg/day (Meng et al., 2019), decreased serum gonadotropin releasing hormone and testosterone at 1.5 µg/kg/day (Wang et al., 2018b), histopathological changes in the hippocampus at 1 µg/kg/day (Wang et al., 2018b), increased biochemical markers of brain inflammation at 1.5 µg/kg/day (Wang et al., 2019a), behavioral changes at 1.5 µg/kg/day (Wang et al., 2019b), and increased liver tumors at 2 µg/kg/day (Xu et al., 2018).

The occurrence of hepatic tumors in mice exposed to microcystin-LR in drinking water for one year (Xu et al, 2018) is particularly notable. Earlier studies (reviewed by WHO, 2003) showed that microcystin-LR can promote the growth of tumors after initiation with a genotoxic carcinogen. The only previous study of microcystin-LR as a complete carcinogen (Ito et al., 1997) found liver tumors after dosing by intraperitoneal injection but not after oral exposure; these results are not definitive because the study did not include an adequate control group and for other reasons. In contrast, Xu et al. (2018) is a well-conducted and well-reported study. Tumors were observed even although the dose groups were small (n=10), and tumor incidence increased in a generally dose-related manner (Control, 0.2 µg/kg/day, and 1 µg/kg/day – 0/10; 2 µg/kg/day – 1/10; 4 µg/kg/day – 3/10; 8 µg/kg/day – 2/10; 16 µg/kg/day – 4/10).

Additional considerations

Díez-Quijada et al. (2019a) reviewed the occurrence and toxicity of microcystin congeners other than microcystin-LR. They report that at least 246 forms of microcystin have been reported. They conclude that microcystin congeners other than microcystin-LR are distributed worldwide and may predominate, and that some of the other congeners may be more toxic than microcystin-LR. However, other forms of microcystin are not considered in the recreational advisory because most of the toxicological data on the effects of microcystins are for microcystin-LR, and other microcystin congeners are not analyzed in recreational waters. Because of the potential for co-exposure to other unidentified forms of microcystin of similar or greater toxicity, a public

health-protective approach is appropriate in development of the Reference Dose and recreational advisory for microcystin-LR.

Conclusions and Recommendations – Microcystin-LR Reference Dose

The recent studies reviewed above support the conclusion that the current NJDEP (2017) Reference Dose for microcystin-LR (0.01 µg/kg/day) is not overly conservative and that the full uncertainty factor of 10 to account for effects at lower doses than the Point of Departure is well-supported.

The LOAELs in all six shorter-term (subchronic or developmental) studies reviewed above and in Table 1 were below the LOAEL of 40 µg/kg/day used as the basis for the NJDEP (2017) Reference Dose. The LOAELs in the three gavage studies were 20-25 µg/kg/day (while noting that effects were reported in a mouse strain that is a model for NAFLD, but not in a comparable strain of wild-type mice in one of these studies).

Three additional drinking water studies found male reproductive effects (with evaluation of different specific endpoints in each study) at LOAELs of 0.2, 2, and 20 µg/kg/day, with the lowest LOAEL of 0.2 µg/kg/day from developmental exposure. The LOAELs of 0.2 and 2 µg/kg/day in two of these studies are 200- and 20-fold lower than the LOAEL (40 µg/kg/day) used for the current Reference Dose.

Finally, the recent longer-term studies provide additional evidence for a variety of toxic endpoints from low doses of microcystin-LR. Most notably, Xu et al. (2018) indicates that microcystin-LR can cause liver tumors in the absence of an initiator.

Based on the above information, no revision to the microcystin-LR Reference Dose of 0.01 µg/kg/day is recommended.

Cylindrospermopsin Reference Dose

Basis of current NJDEP (2017) Reference Dose

The current NJDEP short-term Reference Dose (NJDEP, 2017) for cylindrospermopsin is 0.03 µg/kg/day. It is based on the NOAEL of 30 µg/kg/day for increased relative kidney weight in mice exposed to cylindrospermopsin by gavage for 77 days; this effect occurred at doses of 60 µg/kg/day and above (Humpage and Falconer, 2003). There was no information that could be used to develop a Reference Dose for developmental or reproductive effects, and there was a lack of data on potential immune system and neurological effects. Because of these gaps in the toxicological database, a full uncertainty factor of 10 was applied, and the total uncertainty factor was 1000.

Comparison with USEPA (2015b) Reference Dose

The USEPA (2015b) short-term Reference Dose for cylindrospermopsin is 0.1 µg/kg/day. It is based on the same NOAEL for increased relative kidney weight (Humpage and Falconer, 2003) as the NJDEP (2017) Reference Dose. The difference between the USEPA (2015b) and NJDEP (2017) Reference Doses is due to the difference in the database uncertainty factor to account for data gaps and potentially more sensitive effects. USEPA (2015b) used a partial database uncertainty factor of 3, while, for reasons discussed above, NJDEP (2017) used a full database uncertainty factor of 10.

Additional recent toxicity studies

The PubMed literature search identified only one new study (Díez-Quijada et al., 2019b) of the toxicity of cylindrospermopsin. In this study, male Wistar rats were administered a single dose of 0, 7.5, 23.7, or 75 µg/kg by gavage. It should be noted that the other toxicity studies of cylindrospermopsin reviewed by NJDEP (2017) were conducted in mice, with the exception of one rat study (de Almeida et al., 2013) that used lower doses than Díez-Quijada et al. (2019b). Genotoxicity was evaluated in bone marrow with the micronucleus test and in the comet assay in stomach, liver, and blood, and histopathological examinations were performed on stomach and liver. The percent of micronuclei in bone marrow cells was increased at all doses compared to the controls, but this effect did not increase with increasing dose. The authors state that these positive results in an *in vivo* study confirm earlier reports of *in vitro* genotoxicity. In contrast, the comet assay for DNA strand breaks was negative at all doses in blood, stomach, and liver. Histopathological changes, with severity increasing with dose, were observed in livers and stomachs at all dose levels.

Conclusions and Recommendations for Cylindrospermopsin Reference Dose

The recent study (Díez-Quijada et al., 2019b) reviewed above supports the conclusion that the current NJDEP (2017) Reference Dose for cylindrospermopsin (0.03 µg/kg/day) is not overly conservative and that the full database uncertainty factor of 10 to account for effects at lower doses than the Point of Departure is well-supported. Genotoxicity and histopathological changes were observed from a single exposure to 7.5 µg/kg. This dose is 4-fold lower than the NOAEL of 30 µg/kg/day and 8-fold lower than the LOAEL of 60 µg/kg/day in the 77-day study used as the basis for the current NJDEP Reference Dose.

Based on the above information, no revision to the cylindrospermopsin Reference Dose of 0.03 µg/kg/day is recommended.

Anatoxin-a Reference Dose

Basis of current NJDEP (2017) Reference Dose

The current NJDEP short-term Reference Dose (NJDEP, 2017) for anatoxin-a is 0.1 µg/kg/day. It is based on the NOAEL for lethality of 98 µg/kg/day in mice exposed to anatoxin-a by gavage for 28 days (Fawell and James, 1994; Fawell et al., 1999). The Reference Dose includes a total uncertainty factor of 1000 including a factor of 3 for database gaps regarding developmental, reproductive, and immune system effects and a modifying factor of 3 because the NOAEL is less than 10-fold lower than the LOAEL for lethality in the same study.

Comparison with USEPA (2015c) Reference Dose

The USEPA (2015c) concluded that there are insufficient data to derive a Reference Dose for anatoxin-a at this time.

Additional Recent Toxicity Studies

The PubMed literature search identified no toxicity studies that were not considered by NJDEP (2017).

Conclusions and Recommendation for Anatoxin-a Reference Dose

No revision to the current Reference Dose of 0.1 µg/kg/day is recommended.

CITATIONS

Cao L, Huang F, Massey IY, Wen C, Zheng S, Xu S, Yang F (2019). Effects of Microcystin-LR on the Microstructure and Inflammation-Related Factors of Jejunum in Mice. Toxins (Basel). 11(9).

Chen J, Bian R, Li J, Qiu L, Lu B, Ouyang X (2019). Chronic exposure to microcystin-LR reduces thyroid hormone levels by activating p38/MAPK and MEK/ERK signal pathway. Ecotoxicol Environ Saf. 173:142-148.

Díez-Quijada L, Prieto AI, Guzmán-Guillén R, Jos A, Cameán AM. (2019a). Occurrence and toxicity of microcystin congeners other than MC-LR and MC-RR: A review. Food Chem Toxicol. 125:106-132.

Díez-Quijada L, Llana-Ruiz-Cabello M, Cătunescu GM, Puerto M, Moyano R, Jos A, Cameán AM. (2019b). In vivo genotoxicity evaluation of cylindrospermopsin in rats using a combined micronucleus and comet assay. Food Chem Toxicol. 132:110664.

EFSA (2011). European Food Safety Authority. Guidance on Default assumptions used by the EFSA Scientific Panels and Committee, and EFSA Units in the absence of actual measured data. DRAFT guidance on Default assumptions.
https://www.efsa.europa.eu/sites/default/files/consultation/110707a.pdf

Fawell, J.K and James, H.A. (1994). Toxins from blue-green algae: toxicological assessment of anatoxin-a and a method for its determination in in reservoir water. Report no. FR 0492/DoE 3728 Aug. 1994. Foundation for Water Research, Buckinghamshire, UK.

Fawell, J.K., James, C.P., James, H.A. (1994). Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water. Report no. FR 0395/2/DoE 3358/2 Jan. 1994. Foundation for Water Research, Buckinghamshire, UK

Fawell, J. K., Mitchell, R. E., Hill, R. E. and Everett, D. J. (1999). The toxicity of cyanobacterial toxins in the mouse: II Anatoxin-a. Human and Experimental Toxicology, 18(3): 168-173.

He J, Li G, Chen J, Lin J, Zeng C, Chen J, Deng J, Xie P (2017).. Prolonged exposure to low-dose microcystin induces nonalcoholic steatohepatitis in mice: a systems toxicology study. Arch Toxicol. 91:465-480.

Heinze, R. (1999). Toxicity of cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol. 14: 57-60.

Humpage, A.R., Falconer IR. (2003). Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. Environ. Toxicol. 18: 94-103.

Lad A, Su RC, Breidenbach JD, Stemmer PM, Carruthers NJ, Sanchez NK, Khalaf FK, Zhang S, Kleinhenz AL, Dube P, Mohammed CJ, Westrick JA, Crawford EL, Palagama D, Baliu-Rodriguez D, Isailovic D, Levison B, Modyanov N, Gohara AF, Malhotra D, Haller ST, Kennedy DJ (2019). Chronic Low Dose Oral Exposure to Microcystin-LR Exacerbates Hepatic Injury in a Murine Model of Non-Alcoholic Fatty Liver Disease. Toxins (Basel). 11(9).

Li X, Xu L, Zhou W, Zhao Q, Wang Y (2016). Chronic exposure to microcystin-LR affected mitochondrial DNA maintenance and caused pathological changes of lung tissue in mice. Environ Pollut. 210:48-56.

Meng X, Peng H, Ding Y, Zhang L, Yang J, Han X (2019). A transcriptomic regulatory network among miRNAs, piRNAs, circRNAs, lncRNAs and mRNAs regulates microcystin-leucine arginine (MC-LR)-induced male reproductive toxicity. Sci Total Environ. 667:563-577.

NJDEP (2017). Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin-A. Appendix I of NJDEP (2018). Cyanobacterial Harmful Algal Bloom (HABs) Freshwater Recreational Response Strategy.

Pan C, Chen Y, Xu T, Wang J, Li D, Han X (2018). Chronic exposure to microcystin-leucine-arginine promoted proliferation of prostate epithelial cells resulting in benign prostatic hyperplasia. Environ Pollut. 242 (Pt B):1535-1545.

Sedan D, Laguens M, Copparoni G, Aranda JO, Giannuzzi L, Marra CA, Andrinolo D (2015). Hepatic and intestine alterations in mice after prolonged exposure to low oral doses of Microcystin-LR. Toxicon. 104:26-33.

USEPA (2015a). Health Effects Support Document for the Cyanobacterial Toxin Microcystins. Office of Water. EPA- 820R15102. June 2015.

USEPA (2015b). Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin. Office of Water. EPA- 820R15103. June 2015.

USEPA (2015c). Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A. Office of Water. EPA- 820R15104. June 2015.

Wang J, Chen Y, Chen Z, Xiang Z, Ding J, Han X (2018a). Microcystin-leucine arginine inhibits gonadotropin-releasing hormone synthesis in mice hypothalamus. Ecotoxicol Environ Saf. 2018 Nov 15; 163:391-399. Office of Water. EPA 822-R-19-001. May 2019.

Wang X, Xu L, Li X, Chen J, Zhou W, Sun J, Wang Y (2018b). The differential effects of microcystin-LR on mitochondrial DNA in the hippocampus and cerebral cortex. Environ Pollut. 2018 Sep; 240:68-76.

Wang J, Zhang C, Zhu J, Ding J, Chen Y, Han X (2019a). Blood-brain barrier disruption and inflammation reaction in mice after chronic exposure to Microcystin-LR. Sci Total Environ. 689: 662-678.

Wang J, Chen Y, Zhang C, Xiang Z, Ding J, Han X (2019b). Learning and memory deficits and Alzheimer's disease-like changes in mice after chronic exposure to microcystin-LR. J Hazard Mater. 373:504-518.

Xu L, Li T, Ding W, Cao Y, Ge X, Wang Y (2018). Combined seven miRNAs for early hepatocellular carcinoma detection with chronic low-dose exposure to microcystin-LR in mice. Sci Total Environ. 628-629:271-281.

Zhang H, Wang L, Shen S, Wang C, Xiang Z, Han X, Li D (2017). Toxic effects of microcystin-LR on the development of prostate in mice. Toxicology 380:50-61.

Zhou Y, Sun M, Tang Y, Chen Y, Zhu C, Yang Y, Wang C, Yu G, Tang Z (2020). Responses of the proteome in testis of mice exposed chronically to environmentally relevant concentrations of Microcystin-LR. Ecotoxicol Environ Saf. 187:109824.

Table 1: Recently Published Repeated-Dose Oral Toxicity Studies of Microcystin-LR

Study	*Species, Sex, Strain*	*Duration*	*Exposure Route*	*Exposure Levels*	*Doses* (µg/kg/day)*	*Most sensitive effect(s)*	*NOAEL (µg/kg/day)*	*LOAEL (µg/kg/day)*	*Comments*
Cao et al., 2019	Mouse (sex not specified) - C57Bl/6J	180 days	Drinking Water	0, 1, 30, 60, 90, 120 µg/L	0, 0.2, 6, 12, 18, 24	Histological changes in jejunum (small intestine)	----	0.2	
Chen et al., 2019	Mouse (female) - Balb/c	180 days	Drinking Water	0, 1, 10, 20, 40 µg/L	0, 0.2, 2, 4, 8	Decreased thyroid hormone FT3; increased % apoptotic cells in thyroid	0.2	2	Changes in related hormones (FT4; TSH) at higher doses
He et al., 2017	Mouse (male) - Balb/c	**90 days**	Gavage	0, 40, 200 µg/kg every 2 days	0, 20, 100	Changes in metabolite profiles and liver histology indicative of non-alcoholic steatosis (i.e. non-alcoholic fatty liver disease - NAFLD)	---	**20**	Subchronic study with LOAEL below 40 µg/kg/day used in NJDEP (2016)
Lad et al., 2019	Mouse (male) - Ledpr^{db} (NAFLD model strain); C57Bl/6J (control strain)	**28 days**	Gavage	0, 50, 100 µg/kg every 2 days	0, 25, 50	Biochemical and histological markers of liver damage in Ledpr^{db} strain; no effects in C57Bl/6J	**--- Ledpr^{db}** **50 - C57Bl/6J**	**25 - Ledpr^{db}** **--- C57Bl/6J**	NAFLD increases susceptibility to hepatic toxicity of microcystin-LR Differing results than He et al. (2017) in control strain may be due to differing susceptibility to hepatic toxicity in Balb/c versus C57Bl/6J strains and/or the longer duration of exposure in He et al. (2017)
Li et al., 2016	Mouse (male) – C57BL/6	360 days	Drinking Water	0, 1, 5, 10, 20, 40 µg/L	0, 0.2, 1, 2, 4, 8	Histopathological changes in the lung	0.2	1	

Study	*Species, Sex, Strain*	*Duration*	*Exposure Route*	*Exposure Levels*	*Doses* (µg/kg/day)*	*Most sensitive effect(s)*	*NOAEL (µg/kg/day)*	*LOAEL (µg/kg/day)*	*Comments*
Meng et al., 2019	Mouse (male) – Balb/c	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	% abnormal sperm	0.2	1.5	LOAEL for decreased relative testes wt. – 3 µg/kg/day
Pan et al., 2018	Mouse (male) – Balb/c	**90** and 180 days	Drinking Water	0, 1, 10, 20, 30 µg/L	0, 0.2, 2, 4, 6	Focal hyperplasia of prostate (90 days); more severe changes at 180 days.	**0.2**	**2**	Dose-related increase in biochemical markers of prostate disease
Sedan et al. 2015	Mouse (male) – N:NIH-S	**30 days**	Gavage	0, 50, 100 µg/kg every 2 days	0, 25, 50	Hepatic steatosis (lipid accumulation) and decreased intraepithelial lymphocytes in small intestine	---	**25**	Intraepithelial lymphocytes are involved with immune response in small intestine
Wang et al., 2018a	Mouse (male) – ICR	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	Decreased serum gonadotropin releasing hormone (GnRH) and testosterone	0.2	1.5	Decreases were dose-related; non-significant decreases at 0.2 µg/kg/day
Wang et al., 2018b	Mouse (male) – C57BL/6	360 days	Drinking Water	0, 1, 5, 10, 20, 40 µg/L	0, 0.2, 1, 2, 4, 8	Histopathological changes and effects on mitochondrial DNA in hippocampus and cerebral cortex, more severe in hippocampus	--	1	Severity of histopathological changes in hippocampus increased with dose
Wang et al., 2019a	Mouse (male) – Balb/c	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	Increased glial fibrillary acidic protein (GFAP; marker of astrocyte activation) and TNF-alpha	0.2	1.5	Additional conclusions – microcystin-LR impaired blood-brain barrier and accumulated in mouse brain

						(marker of brain inflammation) in brain			
Study	***Species, Sex, Strain***	***Duration***	***Exposure Route***	***Exposure Levels***	***Doses* (µg/kg/day)***	***Most sensitive effect(s)***	***NOAEL (µg/kg/day)***	***LOAEL (µg/kg/day)***	***Comments***
Wang et al., 2019b	Mouse (sex not specified) - Balb/c	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	Decreased scores on tests of cognitive impairment – "freezing time" in context test, and novel object recognition test	0.2	1.5	Other changes characteristic of Alzheimer's disease including effects on learning and memory, and histological and biochemical changes in the brain.
Xu et al., 2018	Mouse (male) – C57BL/6	90, 180, 270, 360 days	Drinking Water	0, 1, 5, 10, 20, 40, 80 µg/L	0, 0.2, 1, 2, 4, 8, 16	Liver tumors after 360 days exposure. Atypical liver cells after 270 days exposure	1	2	No statistical analysis shown for tumor incidence, but no tumors in controls or lower dose groups. An earlier study (Ito et al., 1997) showing that microcystin-LR caused liver tumors used intraperitoneal injection and did not include a control group.
Zhang et al., 2017	Mouse (male offspring) – Balb/c	**Maternal exposure from gestational day 12 to postnatal day (PND) 21 (~ 29 total days of exposure)** Offspring received *in utero* and	Drinking Water	0, 1, 10, 50 µg/L	0, 0.2, 2, 10 (estimated maternal dose) Potential doses to offspring via ingestion of drinking	In offspring at 90 days of age: Decreased anogenital distance; decreased body wt., decreased relative prostate wt., histopathological changes in the prostate, decreased serum testosterone	----	**0.2**	The effects were clear cut in the lowest dose group (maternal - 0.2 µg/kg/day). LOAEL for effects in offspring at 30 days of age was (maternal) dose of 2 µg/kg/day. It was noted that offspring may have ingested the drinking water directly

		lactational exposure, and possibly exposure via ingestion of drinking water on PND 17-21.			water on PND 17-21 are not known.				on PND 17-21, and that the dose received from drinking water ingestion may have been substantial compared to *in utero* and lactation exposure.
Study	***Species, Sex, Strain***	***Duration***	***Exposure Route***	***Exposure Levels***	***Doses* (µg/kg/day)***	***Most sensitive effect(s)***	***NOAEL (µg/kg/day)***	***LOAEL (µg/kg/day)***	***Comments***
Zhou et al., 2020	Mouse (male), strain not specified	**90** and 180 days	Drinking Water	0, 1, 10, 100 µg/L	0, 0.2, 2, 20	% abnormal sperm tubules	**2 – 90 day exposure** 0.2 – 180 day exposure	**20 – 90 day exposure** 2 – 180 day exposure	Changes in relevant biochemical markers were evaluated at 180 days and were affected by microcystin-LR

NOTE: Study durations, LOAELs and NOAELs from studies with durations of **90 days or less** are shown in **BOLD UNDERLINE** because they are most relevant to development of the short-term Reference Doses used in recreational advisories.

*Doses (µg/kg/day) in studies using drinking water exposure are estimated based on a daily water consumption rate in mice of 0.2 ml/g/day used to estimate the doses in several of the papers that were reviewed. The water consumption rate may have been lower than 0.2 ml/g/day based on estimates for mice provided by EFSA (2011) of 0.18 ml/g/day in subacute studies and 0.15 ml/g/day in subchronic study. If water consumption was lower than 0.2 ml/g/day, the doses (µg/kg/day) would also have been lower than those stated herein.

Background Information on Microcystin "Warning" and "Danger" Threshold Values

NJDEP Division of Science and Research
April 29, 2020

Summary
NJDEP is aware of several states, including California, Ohio, Kansas, and Utah, that have "Danger" (or similar) threshold values for microcystin (shown in table at the end of this document). All of these states also have one (UT) or two (CA, OH, KA) lower tiers of threshold values (e.g. "Advisory", "Warning").

This document provides information to support New Jersey "Warning" and "Danger" threshold values for recreational exposure to microcystin. These higher threshold values will be used along with the lower "Advisory" threshold value to provide tiered advice on recreational exposure to microcystin. These threshold values are summarized in the Table 1 below:

Table 1. Tiered recreational threshold values recreational for microcystin

Recreational Threshold Value	***Microcystin Concentration***
Advisory	**3 µg/L**
Warning	**20 µg/L** *7 times NJ Advisory level based on child exposure.*
Danger	**2000 µg/L** *Child dose would be ~750 times the NJ Reference Dose and ~5 times below NJ LOAEL.* *USEPA (based on WHO) – "very high relative probability of acute health effects."*

NJDEP Microcystin "Warning" Threshold Value

The information below provides support for a microcystin "Warning" threshold value of 20 µg/L.

WHO

WHO (2003) states that an adult (60 kg) who ingests 100 ml of water containing 20 µg/L microcystin while swimming will receive a dose close to the WHO (1998) Tolerable Daily Intake (TDI; equivalent to a Reference Dose) of 0.04 µg/kg/day, and that the health risk would be higher in a susceptible person (e.g. someone with chronic hepatitis B). WHO (2003) also states that a 15 kg child who ingest 250 ml of water during "extensive playing" could be exposed to 10 times the TDI.

The WHO (1998) TDI, 0.04 µg/kg/day, is based on the same mouse study (Fawell et al., 1994) as the NJDEP Reference Dose (0.01 µg/kg/day), but uses an uncertainty factor of 1000 instead of the uncertainty factor of 3000 used by NJDEP. This is because the Point of Departure of 40 µg/kg/day was considered to be a No Observed Adverse Effect Level (NOAEL) by WHO (1998), but it was considered to be a minimal Lowest Observed Adverse Effect Level (LOAEL) by NJDEP based on significant decrease in body weight gain in males, as well as non-statistically significant changes in other parameters (total blood protein, albumin, chronic liver inflammation) that are predictive of significant effects at higher doses. As such, NJDEP included an uncertainty factor of 3 for extrapolation from a minimal LOAEL to a NOAEL that was not included by WHO.

USEPA

Based on information provided by WHO (2003), USEPA (2019a) states that there is a high relative probability of acute health effects from a cyanobacterial bloom capable of producing 20-2000 µg/L microcystin.

Other States

As shown in Table 2 below, two states (CA, OH) use 20 µg/L as a "Danger" threshold value for recreational exposure. Additionally, New York (NYDEC, undated) classifies a HAB with microcystin levels of 10-20 µg/L as "Confirmed with High Toxins Bloom."

Relationship to New Jersey microcystin Reference Dose

WHO (2003) states that a 15 kg child "extensively playing" in water containing 20 µg/L would receive a dose 10 times the WHO (1998) TDI.

Using current NJDEP child recreational exposure assumptions that are based on professional judgement, recreational exposure of a child to water with a microcystin concentration of 20 µg/L would result in a dose 7 times the NJ Reference Dose of 0.01 µg/kg/day.

NJDEP Microcystin "Danger" Threshold Value

The information below provides support for a potential microcystin "Danger" threshold value of 2000 µg/L.

WHO

WHO (2003) states that, when there is a cell count of 100,000 cells/ml, cells can concentrate 100-fold at the surface due to buoyancy to form a "high risk level scum" in the top 4 cm of water that could contain 2000 µg/L microcystin.

USEPA

Based on information provided by WHO (2003), USEPA (2019b) states that there is a very high relative probability of acute health effects from a cyanobacterial bloom capable of producing >2000 µg/L microcystin.

Furthermore, USEPA (2019a) developed a screening analysis for estimation of inhalation exposure near a waterbody contaminated with microcystin, while noting that the estimated exposures are associated with considerable uncertainty. The estimates are based on upper percentile values for daily time spent at a pool, river, or lake from the USEPA Exposure Factors Handbook (USEPA, 2011). Based on the USEPA screening analysis, daily doses from inhalation exposure near a lake with 2000 µg/L microcystin are estimated to be several-fold higher than the NJDEP Reference Dose of 0.01 µg/kg/day.

Other States

As shown in Table 2 below, two states (KA, UT) use 2000 µg/L as a "Danger" threshold value for recreational exposure.

Relationship to New Jersey microcystin Reference Dose

Recreational exposure of a child to water with a microcystin concentration of 2000 µg/L would result in a dose ~750 times higher than the NJ Reference Dose of 0.01 µg/kg/day and only about 5-fold below the minimal LOAEL of 40 µg/kg/day.

Table 2. Other states' Danger (or similar) recreational threshold values for microcystin

State	Advisory	Toxin Level (µg/L)	Cell Count (cells/ml)	Recommended Actions	Basis
California	Danger (Also 2 lower level advisory tiers)	Microcystin >20 Anatoxin-a >90 Cylindro-spermopsin >17	---	Post sign stating that: • There is a present danger. • People, pets and livestock should stay out of the water and away from water spray.	California Cyanobacteria and Harmful Algal Bloom (CCHAB) Network (2016) states: "based on risk management objectives rather than a purely health-based conservative approach" "suggested as a warning level by the World Health

					Organization (WHO 1999)" https://drive.google.com/file/d/13RQyEJ0MB46D6TNN9KpsdFW8pZBZ-Eou/view
Ohio	Danger (Also 2 lower level advisory tiers)	Microcystin >20 Anatoxin-a >300 Cylindro-spermopsin >20	---	• Elevated Recreational Public Health Advisory • Avoid all contact with the water. • Algal Toxins at Unsafe Levels Have Been Detected.	Not provided https://epa.ohio.gov/portals/35/hab/HABResponseStrategy.pdf
Kansas	Waterbody is closed (Also 2 lower level advisory tiers)	Microcystin >2000	>10,000,000	• Recommend that either portions of the lake, the entire lake, or zone, be closed. If necessary – close adjacent land up to 100 ft from shoreline • Post signage* • Notify health dept., doctors, vets, health providers, etc. Post on website* • Issue media release* • Notify public water suppliers* **These actions are also recommended at a less restrictive advisory level.*	Not stated but consistent with 100-fold accumulation in high risk level scum in WHO (1999, 2003) Kansas: https://www.kdheks.gov/algae-illness/index.htm Utah: https://deq.utah.gov/water-quality/harmful-algal-blooms-home
Utah	Danger – High Relative Probability of Acute Health Risks (Also 1 lower level tier)	Microcystin >2000	>10,000,000	• Lake closed • Keep out of the water	

Links to cited documents:

NJDEP (2016) https://www.state.nj.us/dep/wms/bfbm/download/NJHABResponseStrategy.pdf

NYDEC (undated) http://www.dec.ny.gov/docs/water_pdf/habsprogramguide.pdf

USEPA (2019a) https://www.epa.gov/sites/production/files/2019-09/documents/recommend-cyano-rec-water-2019-update.pdf

USEPA (2019b) https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

WHO (2003) https://www.who.int/water_sanitation_health/publications/srwe1/en/