Lake Erie Harmful Algal Bloom Monitoring and Response Strategy

Pennsylvania Department of Environmental Protection
Office of the Great Lakes
Pennsylvania Department of Conservation and Natural Resources
Presque Isle State Park
Erie County Department of Health

Source: NOAA Satellite image 2015
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment Decontamination Between Sampling Locations</td>
<td>25</td>
</tr>
<tr>
<td>Toxin Processing Instructions</td>
<td>25</td>
</tr>
<tr>
<td>Algal Cell Count Processing Instructions</td>
<td>26</td>
</tr>
<tr>
<td>QA/QC</td>
<td>26</td>
</tr>
<tr>
<td>Paperwork</td>
<td>26</td>
</tr>
<tr>
<td>Shipping</td>
<td>26</td>
</tr>
<tr>
<td>APPENDIX D: CYANOTOXIN AND CYANOBACTERIA ANALYTICAL METHODS</td>
<td>32</td>
</tr>
<tr>
<td>Appendix E: HAB Visual Assessment/ Sample Submission Form</td>
<td>35</td>
</tr>
<tr>
<td>Appendix F: Information Notice</td>
<td>36</td>
</tr>
<tr>
<td>Appendix G: Recreational Use Notice</td>
<td>37</td>
</tr>
<tr>
<td>Appendix H: Avoid Contact Notice</td>
<td>38</td>
</tr>
<tr>
<td>Appendix I: Dog Use Notice</td>
<td>39</td>
</tr>
</tbody>
</table>
Acknowledgements
The development of this document could not have been possible without the assistance of the dedicated staff and cooperation of the Pennsylvania Department of Conservation of Natural Resources, Pennsylvania Department of Environmental Resources, Erie County Department of Health, Pennsylvania Sea Grant, Regional Science Consortium, and Citizen Science Volunteers.

1. INTRODUCTION

1.1 Background
Harmful algae blooms have occurred near and around Presque Isle State Park, and elsewhere throughout Lake Erie. Harmful algae blooms can pose a risk to public water supply consumers, recreational water users and their pets. In response, a task force was developed in 2013 to investigate algae blooms and prepare a response strategy. In May 2014, the task force adopted the Lake Erie Harmful Algal Bloom Monitoring and Response Strategy to provide a unified approach to monitor environmental conditions to predict harmful algae blooms, sample for toxin levels in surface waters, use sample results to make advisory decisions, and inform the public and water resource managers about the risks and dangers of harmful algae blooms.

Through the implementation of the May 2014 strategy, it became apparent that changes were necessary to incorporate new scientific information, changes in policies, improved practices and techniques. The May 2014 Strategy remains as the foundation for cyanobacteria and cyanotoxin monitoring and response in Pennsylvania Lake Erie waters, but has been revised and replaced by this July 2017 revision to incorporate the changes made to the program and to streamline access to information for resource management.

1.1.1 Cyanobacteria
Cyanobacteria, commonly known as blue-green algae, are a natural part of the environment and exist in every waterbody on earth. While they are normal, in certain cases they can produce a variety of toxins that can cause illness and death in humans and animals. These cyanotoxins include liver toxins, nerve toxins, and skin toxins. Toxin production is strain specific, and many of these organisms can produce one or several different types of toxins. These toxins are colorless and may persist in the water for some time.

1.1.2 Cyanobacterial Blooms
Cyanobacterial blooms vary in species composition and toxin production over time and within a water body. The distribution is affected by weather and lake conditions, hydrology and morphology. They may be distributed evenly throughout a lake, or may be irregularly distributed because of currents and/or prevailing winds. Areas like shallow bays, coves, sites directly affected by nutrient-rich inflows, or structures that affect flow can significantly affect population growth rates and distribution. Surface scum may develop when cyanobacteria float to
the surface during calm, sunny weather and may dissipate within hours as conditions change.

When a cyanobacteria bloom creates a surface scum, or produces toxin levels that have potential to harm humans or animals, the episode is referred to as a harmful algal bloom, or HAB.

1.1.3 Rationale for Strategy Development
HABs are not rare or unique; they are prolific and predictable in the western basin of Lake Erie and other inland lakes. Although not commonly seen in the central or eastern basins of Lake Erie, HABs have been documented in Presque Isle Bay. Significant cyanobacteria blooms, but not confirmed HABs, have also been observed on inland lakes in northwestern Pennsylvania.

HABs near and around Presque Isle State Park with its 11 public beaches and multiple public access areas to Lake Erie can pose a risk to the 4 million annual park visitors and their pets. HABs are also a concern for other Lake Erie and Presque Isle Bay waters users, among others, public water suppliers, public and private beachgoers, boaters and watercraft users, sportsmen, marina operators, employees working in water environments, and individuals and their pets who come into contact with the water. With the potential public health risk, a comprehensive strategy to provide public education, routine monitoring and posting public advisories in the event of a HAB is necessary to minimize human and pet exposure to cyanotoxins.

1.2 Purpose, Focus and Coordination

1.2.1 Purpose
The purpose of Lake Erie Harmful Algal Bloom Monitoring and Response Strategy is to provide a unified approach to identifying and addressing HABs in Lake Erie waters. The strategy is designed to inform the public and water resource managers on how to recognize a HAB and the associated hazards, monitor environmental conditions to predict HAB formation, monitor toxin levels in surface waters, and use sample results to make public advisory decisions.

1.2.2 Focus
The focus of the Lake Erie Harmful Algal Bloom Monitoring and Response Strategy is on surface waters in Presque Isle Bay and Pennsylvania waters in Lake Erie. Areas near and around Presque Isle State Park are state managed and ensuring the health and safety of the park users is a top priority. Furthermore, Lake Erie serves as a drinking water supply for the Erie community. Another focus of the strategy is to collect information and inform public water suppliers of the environmental conditions and water quality.

Although the focus of the strategy is for Lake Erie waters in Pennsylvania, these same principles and practices can be applied to other public beaches or state managed facilities, and privately managed facilities in or around Presque Isle Bay, Lake Erie, other inland lakes and rivers.

Private ponds and waters sources used for livestock watering are not a focus of this strategy, but
HABs can pose a risk to agricultural operations. Agricultural operators should be aware of the dangers of HABs and how to manage their operation to mitigate threats to livestock.

1.2.3 Coordination
A coordinated effort is necessary to provide public education, monitor environmental conditions that support HAB formation, confirm a HAB episode, advise the public of the hazards associated with HABs, set water use restrictions and respond to HAB related illness. The primary agencies with the responsibility to carry out these actions are the Pennsylvania Department of Environmental Protection (PADEP), the Pennsylvania Department of Conservation and Natural Resources (DCNR) and the Erie County Department of Health (ECDH). The work; however, cannot be completed without the help of other agencies and community partners.

1.3 Agency Responsibilities

1.3.1 Department of Conservation and Natural Resources-Presque Isle State Park
DCNR-Presque Isle State Park managers coordinate the monitoring of state park waters for HAB development, direct responses to HAB related complaints at the park, post appropriate advisories and uses restrictions, and provide informational materials to staff and park users about HABs.

1.3.2 Erie County Department of Health
ECDH consults on public health cyanotoxins threshold levels, advises the public on health hazards of HABs, provides information to the public about HAB safety, makes HAB advisory recommendations, and responds to reports of HAB related illnesses.

1.3.3 Pennsylvania Department of Environmental Protection
PADEP monitors the quality of surface water, conducts and directs research on HAB formation, takes action to reduce nutrient loading into Erie waterways to limit HAB production, trains other partners in sample collection and HAB identification, responds to HAB related complaints in Presque Isle Bay and Lake Erie waters, makes HAB advisory recommendations, and provides informational materials and outreach to the public about HABs.

1.4 Partner Agencies and Organizations

1.4.1 Regional Science Consortium
The RSC is the lead organization for collecting, analyzing and reporting cyanobacteria production and cyanotoxin levels. The RSC also coordinates research on HAB development, advises the task force, and provides HAB informational materials and outreach to the public.

1.4.2 Pennsylvania Sea Grant
PA Sea Grant is the lead organization for communicating HAB awareness risks to the public. PA Sea Grant coordinates HAB training activities, holds workshops and distributes informational
materials to the public. PA Sea Grant also leads efforts to reduce nutrient loading into Erie waterways to limit HAB production.

1.4.3 Citizen Volunteer Monitoring
Citizen scientists conduct sampling of Presque Isle Bay and Lake Erie waters and relay monitoring information to PADEP. They also train private beach and marina managers on HAB identification, advisory protocols, and hazards.

1.4.4 PA Department of Agriculture
The PA Department of Agriculture consults on risks to pets and livestock and recommends animal advisory thresholds, review animal illness reports and provide outreach to the public about HABS.

1.4.5 Supporting Agencies
Several other agencies work in or near surface waters and have routine contact with the public, including the PA Fish and Boat Commission, PA Game Commission and the Erie County Conservation District. These agencies support the task force by reporting suspected HABs, posting advisories at state managed waters, and providing outreach to the public about.

1.5 Review, Reporting and Revision
At the conclusion of each HAB monitoring season strategy implementation will be evaluated by the participating agencies and community partners. The evaluation will include, at a minimum: outreach activities completed, sample collection locations and results, water resource areas of potential concern, confirmed HABs, advisory postings, public response to advisories, and reports of human or pet illness. The results of the evaluation will be compiled into an After Action Report, which will be used as a basis for any modifications to the Lake Erie Harmful Algal Bloom Monitoring Strategy for Recreational Waters. The After Action Report will also be made available for public review.

2. CYANOTOXIN TOXICITY

2.1 Human health impacts from exposure to cyanotoxins
Cyanotoxins have the ability to produce some of the most potent toxins known to humankind. These toxins can affect liver and brain function and can cause severe illness and death. Routes of cyanotoxin exposure include direct ingestion from consuming water containing toxin, and incidental ingestion from swallowing small amounts of water during recreational activities. Incidental ingestion can also result from the inhalation and aspiration during activities where the water containing toxin is aerosolized, such as water skiing or splashing. Cyanobacteria can also cause skin irritation and a rash from the lipopolysaccharides found on cell surfaces, however the toxins are not likely to cross the skin barrier.
Cyanotoxin exposure from fish consumption has been studied and data from multiple sources indicate that toxins mainly accumulate in the organs of fish. Studies conducted by the Ohio EPA, as reported in the State of Ohio Harmful Algal Bloom Response Strategy, have detected microcystin in fillets at low levels.

2.2 Animal health impacts from exposure to cyanobacteria toxins
Animals are also sensitive to cyanotoxins. Exposure to toxins can occur through ingestion when pets and livestock drink water from a contaminated source and by licking their fur after swimming. If toxins are present when animals drink surface water, they can become very ill and even die. Dogs are particularly sensitive and there have been confirmed dog deaths due to cyanobacteria poisoning.

3. CYANOTOXIN AND CYANOBACTERIA THRESHOLDS
There are currently no Pennsylvania or federal human health standards for drinking water or recreational use standards for cyanotoxins or cyanobacteria. The task force reviewed proposed federal guidance, World Health Organization (WHO) publications and multiple state programs to establish exposure thresholds for the strategy.

3.1 Cyanotoxin thresholds for public drinking water
In May 2015 US EPA proposed Drinking Water Health Advisories for two algae toxins, microcystin and cylidrospermopsin. Health advisories are non-regulatory concentrations at which adverse health effects are not anticipated to occur over specific exposure durations. These advisories are based on a 10-day exposure. The task force has adopted the EPA advisory as the drinking water thresholds for the strategy.

3.2 Cyanotoxin thresholds for recreational waters
The Environmental Protection Agency is currently developing Ambient Water Quality Criteria for cyanotoxins in recreation freshwater systems. A draft of the plan is expected to be completed in the fall of 2016. Several states have established cyanotoxin exposure limits and set thresholds to trigger public advisories and water use restrictions. The cyanotoxin thresholds adopted by the State of Ohio are considered by its agencies as protective of human exposures based on information available. The task force has adopted the State of Ohio’s recreational use thresholds for the strategy.

3.3 Cyanotoxin thresholds for dogs
The focus of the strategy includes several areas used by the public for pet activities, including waterfowl hunting, dog water training and dog beaches. The task force recognized the need to notify pet owners of the potential hazards of cyanotoxin exposure. The task force has adopted the Oregon Harmful Algae Bloom Surveillance (HABS) Program dog guidance thresholds for cyanotoxins in recreational water. The dog-specific thresholds are not used as the basis for
public health advisories, but are offered as a guideline for pet owners, water resource managers and veterinarians to use as appropriate.

### 3.4 Pennsylvania Lake Erie cyanotoxin thresholds

This strategy incorporates the recreational use thresholds published by the Ohio EPA, the May 2015 US EPA proposed Drinking Water Health Advisories for drinking water, and the Oregon Harmful Algae Bloom Surveillance (HABS) Program dog-specific guidance thresholds. The thresholds are summarized in Table 1, below.

#### Table 1. Cyanotoxin Advisory Thresholds

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Microcystin (μg/L)</th>
<th>Anatoxin-a (μg/L)</th>
<th>Cylindrospermopsin (μg/L)</th>
<th>Saxitoxin (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recreational Use Advisory</td>
<td>6</td>
<td>80</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Recreational Use Avoid Contact</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Drinking Water Children under 6 years</td>
<td>0.3</td>
<td>-</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>Drinking Water Children over 6 years and adults</td>
<td>1.6</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>Dog Guidance Value</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td>3</td>
</tr>
</tbody>
</table>

(All values expressed as total toxins)

### 3.5 Human Health Cyanobacteria Thresholds for Recreational Waters

An alternative method to determine risks associated with cyanobacteria is to measure the specific type and number of algae cells capable of producing cyanotoxins. The WHO has published guidelines for levels of cyanobacteria cell concentrations that may pose a hazard to human health. The task force has adopted the WHO cyanobacteria exposure thresholds for the strategy, as listed in Table 2, below.

#### Table 2: WHO Guidelines for HABs

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

4.1 Public Water Supplies
Monitoring efforts are focused on areas where there is the greatest risk of human exposure. Public drinking water sources are top priority. On December 11, 2015, the U.S. EPA published the proposed fourth Unregulated Contaminant Monitoring Rule, which will require cyanotoxin monitoring by all large surface drinking water supplies serving more than 10,000 people. Sampling is expected to take place between 2018 and 2020. In the interim, information collected during routine or special monitoring indicating the presence of cyanotoxins at or above drinking water threshold levels listed in Table 1, above, will be shared with public drinking water suppliers and the DEP Safe Drinking Water Program for appropriate response.

4.2 Recreational Areas
The second monitoring priority is recreational areas where there is a high risk of public contact with cyanobacteria or cyanotoxins. Recreational areas identified for HAB monitoring and response activities are prioritized based on the likelihood of public contact and/or ingestion. Water resource managers identify the priority level of a specific location based on local use, season, or special events. Priority levels may change depending on public use and seasons.

- **Priority I** – High risk of public contact– public beaches, canoe/kayak launches, popular wading areas and public drinking water intakes

- **Priority II** – Intermediate risk of public contact– public marinas, docks, open water likely to be used for recreational activities with direct water contact

- **Priority III** – Areas of public waters that represent minimized contact/ingestion risk - public fishing access locations and shoreline walkways with public water access

Certain areas with a high likelihood of pet exposure may also be designated by a resource manager as a priority-designated waterfowl hunting sites, pet training and exercise areas.

4.3 Lake Erie Sampling Plan
The RSC conducts routine monitoring of Presque Isle Bay and state park beaches. Citizen Science Volunteers also assist with the monitoring. Monitoring is focused on cyanobacteria production and cyanotoxin levels. Satellite imagery and a water quality buoy located in Presque Isle Bay may also be used to assist in identifying the location of HABs. The monitoring areas for Presque Isle State Park, Presque Isle Bay and certain areas of Lake Erie are identified in Appendix B.
4.4 HAB Sampling Protocol
Sampling protocols are designed to be responsive to HAB reports so that public health may be protected. The procedural requirements for collecting, preserving, transporting and analyzing phytoplankton and/or cyanotoxin samples are described in Appendix C.

4.5 Analytical Methods

Various methods are available to identify the presence of cyanobacteria and cyanotoxins in surface waters. The specific analytical methods used for the strategy to make public advisories are listed in Appendix D. Note that cyanotoxin results reported for advisory determinations are expressed as total toxins (dissolved toxin and lysed cell toxin).

5. HAB IDENTIFICATION AND REPORTING

The initial observation of a potential HAB involves identifying the presence of color and/or scum in surface waters. Frequent, close monitoring of the bloom’s location(s) should be continued, especially in priority water resource areas. Generally, the water will be discolored if the algae count is 4,000 cells/ml or greater. The color can vary from brown, green, blue green, white, black, purple or red. A HAB is verified by the presence of a cyanobacteria surface scum or through sample analysis for cyanotoxins or algae cell counts. It’s important to note that cyanotoxins may be present without the presence of cyanobacteria.

5.1 Presque Isle Bay and Lake Erie Waters- A report of a potential HAB in Presque Isle Bay and Lake Erie waters, outside of Presque Isle State Park, can be made by notifying the ECDH and PADEP Customer Service Representative at the contact information listed above. Bloom observers can submit digital photographs with the HAB Visual Assessment Data Sheet for HAB evaluation. Close-up (within 24 inches) and landscape photographs showing the extent and location of the bloom are needed for evaluation. If no photographs are available, or a determination cannot be made, agency will visit the site to corroborate the initial report.

5.2 Presque Isle State Park Waters- A report of a HAB on Presque Isle State Park should be directed to a park manager or the park office for response. If park staff are unavailable, or the park office is closed, citizens can make a report by notifying the PADEP Customer Service Representative at 814.332.6839, or by emailing the HAB Visual Assessment Data Sheet (Appendix E) to PADEP’s online complaint report system at www.dep@state.pa/. The report should also be relayed to PADEP and ECDH for coordination.

5.3 Human and Animal Illness
Reports of human illnesses believed to be related to HABs should be relayed to the ECDH. Reports of animal illness should be relayed to the PA Department of Agriculture and the ECDH.
6. HAB COORDINATION AND RESPONSE

A confirmed HAB, whether it is a cyanobacterial scum or sample results that exceed cyanobacteria or cyanotoxin thresholds, should be reported to the responsible agencies to ensure appropriate public notification and posting advisories. The party conducting the monitoring should notify the task force to verify that conditions meet the criteria for a HAB.

6.1 HAB Notification
Email is the main method used to alert agencies and organizations of a HAB event. The RSC sends cyanotoxins sample results to all task force members listing the monitoring location, sample results, comparison to advisory thresholds and recommended advisories. DEP sends information of a verified cyanobacteria scum to all task force members. From this information the responding agency will make an advisory determination and contact the resource manager.

In cases where there is an impact to public a water supply or a widespread HAB event the responsible agencies will issue a local news release. The news release will contain information about the nature and location of the advisory, possible health effects, recommended protective actions and where to obtain more information. A news release will also be issued when advisories are lifted.

6.2 HAB Advisories
Advisories are necessary to inform the public on how to recognize a HAB and the potential health risks associated with exposure to cyanotoxins. Different advisories and action levels are implemented based on the water use priority level and the available evidence.

6.2.1 Public Water Supply Advisories
Water consumption restrictions and public advisories should be based on appropriate drinking water thresholds. The public water supplier is responsible to coordinate and announce public water consumption advisories.

6.2.2 Recreational Use Advisories
The three methods used in this strategy to inform the public of risks and water use restrictions are: Informational Notice, Recreational Use Advisory, and Avoid Contact Advisory.

6.2.2.1 Informational Notice
Informational Notices are educational. Signage will inform water resource users on how to identify HABs, list the associated risks, safety precautions, reporting information, and where to get more information. Informational Notices should be posted in prominent locations throughout the summer season when HAB events are most likely to occur. Posting Informational Notices is the responsibility of the managing agency. A sample of the Informational Notice used for
Presque Isle State Park is located in Appendix F.

6.2.2.2 Recreational Use Advisory
A Recreational Use Advisory should be issued when cyanotoxin levels are equal to or exceed the Recreational Use Advisory thresholds, or the algal cell count exceeds cyanobacteria guidance levels. Recreational Use Advisory signs warn citizens of the hazards associated with HABs. Recreational Use Advisory signage postings are the responsibility of the managing agency. A sample of the Recreational Use Advisory sign is located in Appendix G.

6.2.2.3 Avoid Contact Advisory
An Avoid Contact Advisory should be issued when there is a visible cyanobacteria algae surface scum, or when cyanotoxin levels and/or algae cell counts are equal to or exceed the Avoid Contact Advisory threshold. The Avoid Contact Advisory sign will be posted to warn citizens that high levels of algal toxins have been detected and to avoid contact with the water. A sample of the Avoid Contact Advisory sign used for Presque Isle State Park is located in Appendix H. Avoid Contact Advisory signage postings are the responsibility of the managing agency.

6.2.3 Dog Use Notice
Water resource managers that oversee areas used for dog water recreation and training should provide information to the users about the risks of HABs to pets. Because of the difficulty in determining the cyanotoxin levels at any particular time, areas used for dog water recreation that are prone to HAB formation should be posted with Informational Notices throughout the season. A Dog Use Notice may be used by the water resource manager in these areas when cyanotoxin levels are equal to or exceed the Dog Guidance value or when a visible scum is present. A sample of the Dog Use Notice sign is located in Appendix I. Dog Use Notice signage postings are the responsibility of the managing agency.

6.2.4 “All Clear”
HAB advisories should remain in place until verification is made indicating that conditions are below recommended thresholds. An “All Clear” notice may be issued at Priority I areas only after confirmation through cyanobacteria and cyanotoxin analysis. An “All Clear” notice may be issued at priority II and III areas either after confirmation through cyanobacteria and cyanotoxin analysis, or after surface scum and bloom is absent for one week.

HAB “All Clear” notice recommendation is made by PADEP and ECDH, and is administered by the water resource manager. In certain case an “All Clear” for recreational use may be issued, but a dog advisory may need to remain in place depending on cyanotoxins levels.

6.3 HAB Response Procedure
The general procedure for HAB response is confirmation, notification, posting advisories and continued monitoring until “All Clear”. The procedure is illustrated below and in Figure 1.

**General Presque Isle Bay/Lake Erie Response Procedure**

Report of cyanobacteria surface scum:
- DEP response and verification
- Task Force notification
  - ECDH - Priority based advisory determination
  - Resource Manager - post Avoid Contact Advisory, monitor

Cyanotoxin or cyanobacteria results exceed Recreational Use/Avoid Contact thresholds:
- RSC initiates Task Force notification
  - ECDH - Priority based advisory determination
  - Resource Manager - post Recreational Use Advisory, monitor

Cyanotoxin results exceed Dog Guidance Value:
- RSC initiates Task Force notification
  - ECDH - Guidance notification
  - Resource Manager - Recreational Dog Use Advisory, monitor

**Presque Isle State Park Specific Response Procedure**

Confirmed cyanobacteria surface scum:
- All Priority areas-post Avoid Contact Advisory

Cyanotoxin or cyanobacteria results exceed Recreational Use thresholds:
- All Priority areas-post Recreational Use Advisory

Cyanotoxin or cyanobacteria results exceed Recreational Use Avoid Contact thresholds:
- All Priority areas-post Recreational Use Advisory

Cyanotoxin results exceed Dog Guidance Value
- All Priority areas-post Dog Use Advisory
<table>
<thead>
<tr>
<th>Threshold</th>
<th>Microcystin (μg/L)</th>
<th>Anatoxin-α (μg/L)</th>
<th>Cylindrospermopsin (μg/L)</th>
<th>Saxitoxin (μg/L)</th>
<th>Cell Counts (cells/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recreational Use Advisory</td>
<td>6</td>
<td>80</td>
<td>5</td>
<td>0.8</td>
<td>20,000</td>
</tr>
<tr>
<td>Avoid Contact Advisory</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>3</td>
<td>100,000 or scum present</td>
</tr>
<tr>
<td>Dog Use Advisory posted at manager’s discretion</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. HAB Monitoring and Response Flowchart
REFERENCES


APPENDIX A: GLOSSARY

Algal toxin: A toxin produced by cyanobacteria. Also called cyanotoxin.
Anatoxin-a: A nerve toxin produced by a number of cyanobacteria.
Beach: Area along the shore that is a designated swimming area and is managed for public use.
Biovolume: Biovolume can be estimated by associating the phytoplankton with similar geometric forms and determining the volume of these by measuring the linear dimensions required for its calculation under the microscope (Vadrucci et al. 2007).
Blue-green algae: Photosynthesizing bacteria, also called cyanobacteria (see definition below).
Contact recreational area: Water area where swimming, wading, diving, jet skiing, water skiing, tubing, wakeboarding, windsurfing, kite boarding or any other in-water activity may occur that is likely to result in immersion or ingestion of water.
Cyanobacteria: Also called blue-green algae. These photosynthesizing bacteria may produce toxins that can cause sickness and possibly death in exposed populations of humans and animals. Cyanobacteria can be present as unicellular, colonial, or filamentous organisms. Some have the ability to fix nitrogen and/or regulate their buoyancy.
Cyanobacteria Scum: An accumulation of algae cells on the surface of the water covering an area that is visible and distinctively apparent.
Cyanotoxin (algal toxin): Toxin produced by cyanobacteria. These toxins include liver toxins, nerve toxins and skin toxins.
Cylindrospermopsin: A nerve toxin produced by a number of cyanobacteria.
ELISA (Enzyme Linked Immunoassay): A rapid assessment method commonly used to detect microcystins, cylindrospermopsin and saxitoxin.
ECDH: Erie County Department of Health
Grab Sample: A sample of river, stream, or lake water collected for the purpose of analyzing its constituent water chemistry and/or biological community
HAB (Harmful Algal Bloom): A visually identified concentration of cyanobacteria that discolors the water, or a cell count greater than 4,000 cells/ml of cyanobacteria genera (Shambaugh and Brines, 2003) Accumulations of cyanobacteria cells may be present at the water surface, at a defined depth, or throughout the water column.
Microcystin: A common type of cyanotoxin that is toxic to the liver. There are more than 80 congeners (forms) of this toxin. Microcystin-LR is the most toxic congener.
PADEP: Pennsylvania Department of Environmental Protection
DCNR: Pennsylvania Department of Conservation of Natural Resources
PISP: Presque Isle State Park
Photic zone: The uppermost layer in a body of water into which light penetrates in sufficient amounts to influence living organisms, especially by permitting photosynthesis.
Public Lake: A lake managed by a political subdivision of the State of Pennsylvania.
Saxitoxin: A nerve toxin produced by a number of cyanobacteria.
### APPENDIX B: PA LAKE ERIE SAMPLING SITES

Pennsylvania Lake Erie Site Names and Locations for Harmful Algal Bloom Monitoring by the Regional Science Consortium

<table>
<thead>
<tr>
<th>#</th>
<th><strong>Beach Sites</strong></th>
<th><strong>Coordinates</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zone 1 (Beach 1 West Extension)</td>
<td>42° 6'49.92&quot;N, 80° 9'20.00&quot;W</td>
</tr>
<tr>
<td>2</td>
<td>Zone 2 (Beach 6)</td>
<td>42° 8'39.41&quot;N, 80° 8'17.99&quot;W</td>
</tr>
<tr>
<td>3</td>
<td>Zone 3 (Mill Road Beach)</td>
<td>42° 9'45.33&quot;N, 80° 7'14.87&quot;W</td>
</tr>
<tr>
<td>4</td>
<td>Zone 4 (Beach 9)</td>
<td>42° 10'11.99&quot;N, 80° 6'19.36&quot;W</td>
</tr>
<tr>
<td>5</td>
<td>Zone 5 (Beach 11)</td>
<td>42° 9'45.45&quot;N, 80° 4'42.81&quot;W</td>
</tr>
<tr>
<td>6</td>
<td>Zone 6 (Beach 2)</td>
<td>42° 7'49.37&quot;N, 80° 8'47.08&quot;W</td>
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<tr>
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<td>8</td>
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<tr>
<td>9</td>
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<td>42° 8'13.13&quot;N, 80° 8'26.92&quot;W</td>
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<tr>
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<td>Ferry Slip</td>
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</tr>
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<td>42° 9'16.15&quot;N, 80° 7'8.82&quot;W</td>
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<td>42° 8'43.39&quot;N, 80° 7'50.40&quot;W</td>
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<td>Erie Yacht Club</td>
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<td>Garrison Run</td>
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<td>South Pier</td>
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<td>Lake Cliff Boat Launch</td>
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<td>Elk Creek</td>
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<td>Walnut Creek</td>
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APPENDIX C: HAB SAMPLING PROTOCOL

Safety Precautions
Safety must come first when sampling HAB toxins. Shoulder-length gloves should be worn when sampling HABs. Chest waders should also be worn if collecting a cyanotoxin sample when wading off the shore to protect skin from contact with toxins. A personal flotation device should also be worn. Avoid inhaling spray from boats, wind, or irrigation water from areas with harmful algal blooms. If the sampler is likely to encounter aerosolized cyanobacteria from the water body, goggles and a mask should be worn to prevent contact with spray. Do not ingest or allow the water to come in contact with the skin. Always wash hands with clean, fresh water after sampling and do not touch hands to mouth or other exposed areas of the body before washing. All equipment, gloves, and waders should be rinsed with de-ionized water (not lake water) after collections.

Sampling Methodology Goal
The goal of sample collection in response to a potential HAB report is to verify a HAB episode. Sample collection procedures are focused on obtaining a full representation of area being sampled. Higher cyanotoxin concentrations are expected near shore, especially on the downwind (away from where the wind is coming from) side of a lake. Highest cyanotoxin concentrations are usually expected with scums (below the dead material at the surface), and within dense cyanobacteria blooms. Most cyanobacteria that produce cyanotoxins hold them within their cells and release the toxins upon cell death. Higher cyanotoxin concentrations may be detected after a rapid bloom die-off, such as when algaecide is added to a dense bloom of cyanobacteria producing cyanotoxins.

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

Sample Frequency
Sampling will occur on a case-by-case basis depending on Priority Level and water conditions. Routine sampling will occur during the peak recreational season between Memorial Day and Labor Day. Weekly sampling, or more frequently, should be conducted for suspected or confirmed HABs. Continued monitoring may occur beyond the peak recreational season based on environmental conditions and relative health risk, in consultation with PA DEP, ECDH, PA DCNR, PA Sea Grant, and Regional Science Consortium.
Preparations
Cyanotoxin samples need to be analyzed within 36 hours of collection, and must be kept cold and in the dark. Phytoplankton samples should be kept on wet ice or ice packs, but not frozen.

IMPORTANT – Samples to be shipped to PADEP lab should be sent Monday – Thursday to ensure for ample processing time. Be sure to contact the DEP sample courier with any questions before shipping.

Label Information
Label the collection containers with a waterproof marker or attach a label to the outside of the container and mark with a waterproof marker. Include the following information:
*Site, Name, Date, Time, Preservative (if applicable)*

Sample Collection

Phytoplankton Sample Collection
The purpose of collecting phytoplankton samples is to identify organisms to determine if the bloom consists of cyanobacteria. If the bloom is cyanobacteria, then the genus of cyanobacteria will determine which cyanotoxins should be analyzed. If the location of the bloom is evident (i.e. at the surface, just below the surface, or benthic), collect a grab sample from the densest part of a bloom. Wear protective gloves when collecting these samples. The grab sample should be collected in a 500ml Nalgene bottle or other PADEP-approved container. If the bloom is not at a distinct location, but diffuse throughout the water column, use a composite sampler that includes a collection for a range of depths.

Ideally, samples should be preserved at the time of collection with Lugol’s iodine solution at a ratio of 1:100, although Lugol’s can be added to a sample anytime within eight hours. To achieve a 1:100 ratio add about 1 ml of Lugol’s solution per 100 ml of sample. Final preserved sample color should be similar to that of weak tea. Deliver samples to the PADEP at TREC for shipment to the PADEP main laboratory in Harrisburg. Samples should be kept on wet ice and in the dark during transport. **Do not freeze the phytoplankton sample - doing so will make identification difficult.**

Cyanotoxin Sample Collection –Overview
Samples collected for cyanotoxin analysis should be analyzed the (Enzyme-linked Immunoabsorbant AssayELISA analysis Regional Science Consortium for a more comprehensive. These will be collected from a suspected HAB using a composite sampler (Appendix A) at nine locations within the designated recreational area and composited. The nine locations will be determined by evenly dividing the recreational area into three transects that begin at the beach and extend into the water. Samples will be collected from three locations (ankle, knee and hip deep) along each transect. Note: use a rod ahead of where you are walking
to gauge depth. Do not stir up the sediment. If the depth drops off quickly past hip depth, then collect ankle-depth and knee depth samples. Do not go past hip depth. Wade slowly (as not to stir bottom substrate) to the sampling locations. Avoid collecting suspended sediment that may be kicked up while accessing the sampling point. Ankle-deep water samples will be collected approximately 15 cm below the surface. Knee- and hip-deep water samples will be collected approximately 30 cm below the surface. If dense cyanobacterial accumulations are present outside of the transect locations (which includes a scum or heavy biomass in the photic zone), an additional sample will be collected from the densest accumulation by filling a separate clean 500ml Nalgene sampling bottle or other PA DEP approved container half way (250 ml). Submit this sample in addition to the composited samples with a separate Sample Submission Form and clearly marked as scum (adapted from USGS, 2008).

**Cyanotoxin Sample Collection Instructions**

1) Use a clean 500ml Nalgene sampling bottle or other PA DEP-approved container to collect from each sampling point along all three transects at a Priority I location. Carry the clean bucket with you (or you can place a float around the bucket). Fill the 500ml Nalgene sampling bottle or other PA DEP-approved container from the ankle-depth location on the first transect and completely dispense the collection into the bucket. Carefully wade out to the knee-depth location with the bucket and collect another 500 mL sample using the same Nalgene sampling container or other PA DEP-approved container. Completely dispense the sample into the bucket. Then wade out to hip depth and collect another 500mL sample and completely dispense the collection into the bucket.

2) Go to the second transect. Using the same 500ml Nalgene sampling bottle or other PA DEP-approved container, collect the three samples along the second transect in the same way the samples were collected along the first transect and dispense them into the bucket with the first transect collections. Once the second transect collections are dispensed into the bucket, go to the third transect and collect the three samples along the third transect in the same way collections were made on the first two transects and dispense into the bucket.

3) Use a clean stirring rod to mix the composite samples from all three transects in the bucket. Continue to stir the composite sample while you dispense a sub-sample of the composite sample into the same 500ml Nalgene sampling bottle or other PA DEP-approved container you used to collect all the samples at that beach. This is the sample you will submit to the laboratory.

4) In addition, if a scum is found at any area where the public is expected to recreate outside the transect lines, collect a surface grab sample which includes the scum at the scum-water interface and clearly noted on the container label. Note the percentage of recreational area covered by the scum on your Sample Submission Form. This sample is not mixed into the composite sample but submitted to the laboratory in addition to the composite sample.
5) Immediately transfer each capped sample to a dark cooler on wet ice or ice packs when collected. The sample must be kept in the dark and cool to preserve any toxin that may be present.

If there are multiple Priority I locations on a single lake with cyanobacteria blooms, all beaches should be sampled in the same manner as stated above, differentiating each sample location by an alternate location name. When you move to a new beach location to set up new transects, rinse the collection bucket and stirring rod three times with lake water at each location. Rinse away from the transect sampling points so as not to cross contaminate or mix the water where samples will be collected. Use a new, clean 500ml Nalgene sampling bottle or other PA DEP-approved container for each different beach sampled.

Open Water (Inland Lakes)
Open water sampling is not prescribed by this Strategy, but if it is deemed necessary, this section describes the methodology for collecting samples.
Establish a central sampling point in the approximate center of a HAB on the open lake and record the latitude and longitude. Each time an open-water HAB sample is collected, there will probably be a different central sampling location and those coordinates should be recorded each time. Collect phytoplankton and toxin samples.
Choose one of the following methods that will best capture the extent of the HAB.

Radial Transect Method (for irregular-shaped, or elongated HABs)
Project three transects through the central sampling point ensuring there are six equal arcs radiating from the central sampling point. Extend each of the six radial arms to the shore. Each of the six radial arms, divide each into two equal length segments with two equally spaced sampling points (not counting the central sampling point.)

Phytoplankton Samples
Using a vertical-composite sampler, collect a phytoplankton sample from the densest bloom area and dispense the sample into a 500ml Nalgene sampling bottle or other PA DEP-approved container or a clean bucket. Take a 500ml Nalgene sampling bottle or other PA DEP-approved container or sub-sample for analysis. Collect additional separate samples of blooms that have a different appearance if applicable and note the latitude and longitude of each collection. Note if a scum is included in the collection. Preserve with Lugol’s iodine (1 ml Lugol’s solution to 100 ml sample). Do not freeze sample.

Cyanotoxin Samples
Collect a grab toxin sample in a rinsed 500ml Nalgene sampling bottle or other PA DEP-approved container at each collection point. Rinse by filling the 500ml Nalgene sampling bottle or other PA DEP-approved container with native water on the opposite side of the boat from where the collection will be made. Collect 1 quart sample from the photic zone where there is the highest concentration of cyanobacteria at each sampling location. If there is a surface scum,
collect a surface sample (scum-water interface) at that location. If it is unclear where the highest concentration of phytoplankton is located in the water column, then collect a grab sample from approximately 15 cm below the surface. Combine a sample collected from the central sampling point to the 12 sample collections along each of the six radial arms in a clean churn splitter or clean bucket. Mix the composite sample in the churn splitter or in the bucket with a clean stirring rod and continue to mix while decanting a sub-sample into the 500ml Nalgene sampling bottle or other PA DEP-approved container. If saxitoxin analysis is ordered, collect a sample form the churn splitter or clean bucket in a 40 ml glass vial pre-dosed with preservative from DEP.

**Important** - The composite sample should be placed on wet ice or ice packs in a cooler as soon as possible.

**Perpendicular Transect Method (For regular-shaped, or round HABs)**

Establish two transects that cross at right angles through the central sampling point. Extend each transect end to the shore. Along each of the four radial arms, divide each into three equal length segments with three equally spaced sampling points (not counting the central sampling point.)

**Phytoplankton Samples**

Using a vertical-composite sampler, collect a phytoplankton sample from the densest bloom area and dispense the sample in a clean bucket. Collect a subsample in a clean 500ml Nalgene sampling bottle or other PA DEP-approved container. Collect additional separate samples of blooms that have a different appearance if applicable and note the latitude and longitude of each collection. Note if a scum is included in the collection. Preserve with Lugol’s iodine (1 ml Lugol’s solution to 100 ml sample).

**Cyanotoxin Preservation Instructions**

Upon collection, samples should be immediately put in a cooler in the dark and on wet ice. If a sample will not arrive for processing at the laboratory within 24 - 36 hours, the sample must be frozen in a standard freezer until it is processed.

**Phytoplankton Preservation Instructions**

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

**Equipment Decontamination Between Sampling Locations**

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

**Toxin Processing Instructions**

Total toxin (dissolved-phase toxins and cell-bound toxins) shall be determined for recreational
water sample analysis. Samples will be processed to ensure all algal cells are lysed. Cells may be lysed utilizing freeze thaw cycling or chemical lysis (i.e. Abraxis Quicklyse).

**Algal Cell Count Processing Instructions**
Samples should be analyzed for cyanobacteria cell count using the…

**QA/QC**
PA DEP will use quality assurance/quality control procedures that meet quality objectives for HAB sampling.

**Paperwork**
Fill out a HAB Visual Assessment/ Sample Submission Form (one for each sample) (see attached templates in Appendix E). Put the paperwork in double ziplock-type bags and seal each bag well. Place the paperwork on the samples in the cooler.

**Shipping**
Contact the appropriate laboratory prior to collecting samples. Include any paperwork required by the receiving laboratory. Make sure that the data are reported back to the sample submitter and to the HAB Coordinator so that data can be entered into the HAB database. Enclose each sample container in a separate sealed plastic bag. Place on ice in a sealed plastic bag and place in the shipping container. Note that ice packs should be used if shipping Fed-Ex and wet ice sealed in plastic bags or ice packs for UPS shipments. Prepare the package for shipment.
SAMPLING AND SAFETY MATERIALS

Materials for basic grab sample phytoplankton and toxin collections at beaches:
- Plastic shoulder-length gloves (to protect skin from toxin irritation)
- Goggles, and mask for over nose and mouth
- Chest waders – if collecting samples by wading off the shore
- Personal flotation device (PFD)
- For phytoplankton collections: Two 500ml Nalgene bottles or other PA DEP-approved containers/beach to sample (one for phytoplankton and one for additional scum sample if needed)
- For toxin collections: Two 500ml Nalgene bottles or other PA DEP DEP-approved containers/beach to sample (one for transect collection which is used for the final composite collection and one for a scum sample (if any) outside the transects)
- 40 ml vials from DES pre-dosed with preservative for saxitoxin collection (if ordered by HAB Coordinator)
- Secchi disk (if available)
- Lugol’s iodine
- Clean bucket (at least 12 quart capacity) and clean non-porous stirring rod (metal or plastic, not wood)
- Centimeter measure for selecting sampling depth at sampling locations
- Walking stick to check depth ahead of sampling when wading
- Yardstick or weighted measuring tape
- Digital camera to record appearance of bloom (submit to HAB Coordinator)
- GPS or a map to mark the location of collection (email scanned map to HAB coordinator)
- Cooler with wet ice or ice packs
- Waterproof permanent marker
- Large trash bags and twist ties (to contain ice in cooler)
- Sample Submission Forms (See Appendix E)
- FedEx or UPS shipping labels
- Multi-probe sampler (if available)

* Collection containers and preservative will be determined by PA DEP.
For composite phytoplankton collections, add the following:

- Vertical whole water composite sampler (2 m integrated tube sampler)
- Build the units as a two-piece for easier transport, although they may be built as a one-piece as well. The sampler is constructed from 1 1/4 inch Schedule 40 PVC pipe and fittings. The graduations on the sampler are in tenth meter increments which can be marked with colored tape. The material list is:

1. one 1 1/4 inch neoprene stopper
2. one PVC coupler (slip to slip type)
3. 1 meter of 1 1/4 inch PVC pipe
4. one 1 1/4 inch valve (optional; can also be accomplished with a stopper if necessary) (Modeled after Minnesota DNR. Not commercially available)

- Churn splitter OR clean bucket (at least 16 quart capacity) and clean non-porous stirring rod (metal or plastic, not wood)
- Multi-probe sonde/water quality instrument (if available)
- At least two 500ml Nalgene bottles or other PA DEP-approved container for phytoplankton collection(s)
- At least two 500ml Nalgene bottles or other PA DEP-approved container for toxin collections
- Lake map or chart to measure off transects
- Boat
1 meter long – 1\(\frac{1}{4}\)” PVC

Rubber Stopper

Connector piece to accommodate the stopper

Stopcock Valve (such as used for a carboy)
HAB VERIFICATION

STEP 1 Examine the material visually:
NOT cyanobacteria if:
- you can see leaf-like structures or roots
- the material is long and stringy, or can be lifted out of the water on a stick
- if it is firmly attached to plants, rock or the bottom (e.g. you can’t lift it out)
MAY be potentially hazardous cyanobacteria if :
- the material consists of small particles that are pinhead size or smaller
- the material is collecting in a layer at the surface or along the shoreline
- the water is murky and colored a brownish green, milky green or blue

STEP 2 Do the “float” test:
Many cyanobacteria can regulate their buoyancy and will float to the top of the water when it is calm. Most other algae don’t have this ability. Most debris and plant material will sink or be identifiable as debris. Microscopic animals will swim randomly and often with a jerky motion.
You can check to see if cyanobacteria are present by filling a clear 2L soda bottle or a bucket with water. The water should be collected away from any debris or large plant material floating along the shoreline. Allow the bucket or bottle to stand in a quiet sunny place, out of the wind. If present, cyanobacteria will often begin to move toward the surface. Wait 15 – 30 minutes and observe the upper portion of the container. Cyanobacteria, which may be a mix of several different kinds, will tend to accumulate in the upper portion while debris and plant material will be at the bottom. There may be smaller material in the middle, which will remain suspended for some time. When filling the container from a dense accumulation, minimize skin contact with the material by wearing gloves or a plastic bag over your hands.
A thin layer of cyanobacteria at the top is usually not a problem. Cyanobacteria are found in most water bodies at concentrations that are not of concern. If you have ruled out non-cyanobacteria using the steps above, and there is a thick layer that is more than an inch deep at the top of the container, it may be prudent to have the sample examined microscopically. Be aware that the concentration of cyanobacteria at a location can change daily, even hourly, as the weather conditions change. If you do the float test routinely, you will begin to become familiar with how the water and cyanobacteria look under different conditions. Also, cyanobacteria may not always move to the surface in 30 minutes. If there is a bloom in progress, with a large amount of cyanobacteria in the water, at least a portion should move toward the surface. With experience, you will become familiar with how your lake looks and when conditions warrant a closer examination.
APPENDIX D: CYANOTOXIN AND CYANOBACTERIA ANALYTICAL METHODS

ELISA protocol for cyanotoxin detection

Microcystins-ADDA ELISA Standard Operating Procedure

This procedure is for the Abraxis Microcystins/Nodularins (ADDA) ELISA Kit

QuikLyse Procedure:

1. Label 2 sets of sterilized glass vials with respective sample IDs. You will need one vial to complete the lysing, and one vial to store filtered lysed sample.
2. Place all glass vials in Styrofoam test tube holder.
3. Transfer 1 mL of sample to each respective vial. Only place sample in ONE of the glass vials in the set designated for each sample.
4. Transfer 100 µL of Reagent A to each vial holding sample. Replace the caps on the vials.
5. Shake the Styrofoam test tube holder for 2 minutes.
6. Allow the samples to incubate at room temperature for 8 minutes.
7. Add 10 µL of Reagent B to each vial holding sample.
8. Repeat steps 5 and 6.
9. Using sterile disposable pipettes and wearing gloves, pipette most of the liquid from the glass vial.
10. Place the filtration pipette tip on the end of the disposable pipette. Gently squeeze the top of the disposable pipette and filter the liquid through the filtration tip into the corresponding glass vial (the one that hasn’t been used yet).

Running the Test

1. Kit is to be stored in refrigerator, but the kit must reach room temperature before a test is performed. It is best to remove the kit from the refrigerator 30-45 minutes prior to use.
2. Make wash buffer by adding 20mL of 5x concentrate to 100mL distilled water (or more as needed). Don’t make excessive amounts of wash buffer.
3. Make a grid of the plate layout so wells can be accounted for. Each well is only good for 1 test.
4. Remove wells that will not be used in this test by popping them out of the plastic microtiter plate. Store them in the sterile bag that the plate came out of until they are needed. Be sure to wear gloves while removing the wells to avoid contamination.
5. Remove the foil linings that protect the caps of the control and standards. Be sure to handle all controls and standards while wearing gloves. These solutions contain sulfuric acid which is a skin and eye irritant.
6. Add 50µL of the control, each standard solution (0-5). Be sure to include a double of each control and standard. Change pipet tips between each standard.
7. Add 50 µL of each sample to wells according to the grid you have created. Make sure the pipette tip is changed between each.
8. Include two more controls (one and a duplicate) at the very end of all of the samples.
9. Add 50µL of the antibody solution to all wells.
10. Cover wells with parafilm and move the plate in a figure eight motion on bench top for 30 seconds.
11. Incubate at room temperature for 90 minutes.
12. Lay out several layers of paper towels in the bench top within a tray. Decent the contents of all wells onto the paper towels by turning the plate upside down and patting it gently on the towels.
13. Wash the wells 3 times with 250µL of wash buffer solution.
14. Lightly pat inverted plate on a new set of paper towels after each wash.
15. Add 100µL of the enzyme conjugate solution to all wells.
16. Cover wells with parafilm and move the plate in a figure eight motion on bench top for 30 seconds.
17. Incubate at room temperature for 30 minutes.
18. Remove parafilm and decant content of wells onto a new set of paper towels.
19. Wash the wells 3 times with 250µL of wash buffer solution.
20. Lightly pat inverted plate on a new set of paper towels after each wash.
21. Add 100µL of substrate (color) solution to wells.
22. Cover with parafilm and shield from light. Incubate at room temperature for 20-30 minutes.
23. Add 50µL of stop solution to wells.
24. Read the plate within 15 minutes of addition of stop solution.

**Reading the Plate**

1. Turn the power on to the Epoch ELISA reader and let it warm up for 2 minutes. Power switch is on the bottom right.
2. On the desktop open the Gen5 2.05 software.
3. Click on Mycrocystins2014.prt
4. Change the plate layout so the wells are labeled accordingly to the grid you have created. This can be done by clicking on the symbol at the top of the software the looks like a blue plate with a small orange circle on it. Choose the desired well label from the left hand side and then click the appropriate place on the well grid that corresponds with your handmade grid sheet.
5. Read the plate by clicking the green play button. A buzzing sound will occur as the Epoch machine reads the plate.
6. Open a new Excel document. Export each matrix from the Gen5 software to Excel by clicking the green export button located on the grid. You should include the grid, standard line graph, and any other data that is produced by the software in the Excel
document. Save as “Abraxis Microcystins Raw Data mm/dd/yy”
7. Open the Abraxis Microcystins Template.
8. Copy and paste the absorbances from each standard into the corresponding locations at the top of the Abraxis sheet (gray boxes only).
9. Below the graph in the Abraxis sheet, list the sample ID’s and then copy and paste the absorbances from the raw data sheet to the Abraxis sheet for each sample respective to its ID on the sheet (gray boxes only).
10. Go to the data tab, click the Solver button, and click okay until you are out of the solver.
11. If an error message occurs from the solver, place the number “1” into the bright yellow boxes and solve again.
12. The solver aligns the curve on the graph with the standard absorbances. All concentrations of toxin in each sample are calculated from this curve.
13. The worksheet calculates the concentration of Microcystins toxin in ng/mL, which is equivalent to µg/L. The concentration for each sample is calculated, and the average between the two is taken. Be careful to read the data from the correct column. If the sample was not completed in duplicate, the average is inaccurate.
14. The kit detects concentrations down 0.1 µg/L. Therefore, detection of 0.1 or below, including a negative is technically a zero detection.
15. The drinking water threshold value is currently set at 0.3 µg/L for children and 1.6 µg/L for adults. Notify Erie Water Works if concentration value exceeds either of these limits.
16. The recreational advisory thresholds are currently 6 µg/L for a recreational public health advisory and 20 µg/L for a recreational no contact advisory. Notify superiors if any of the bay or beach samples exceed these thresholds.

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5. Karen Tobin: KTobin@eriecountypa.gov

Cell Count Method
Samples analyzed for cell count should follow the PA DEP’s BOL7018 Plankton Identification and Enumeration protocol. A Yellow Springs Instrument’s phycocyanin sensor will be used in the field as a proxy for cell counts when samples are unable to be sent to the lab.
Appendix E: HAB Visual Assessment/ Sample Submission Form

Date of Assessment: ________________
Name of Waterbody: ____________________________________________
Location (as specific as possible, town, beach name or other easily identifiable landmarks nearby):
________________________________________________________________
Coordinates: _________________   _________________
Report Completed by: __________________________
Existing Advisory Level: ______Priority Level (I, II, or III): ______
Weather Conditions (sunny, rainy, approximate air temperature):
________________________________________________________________
Water Temperature: ______
Wave Conditions: ______
Water Clarity (circle all that apply):  Clear  Cloudy  Hazy  Opaque  Don’t know
Water Color: ________________________________
Visible Scum: ______
If Buoyancy Test is completed, thickness of a scum (mm/time):_____/_____
Water usage at this location (check all that apply):
□ Swimming or other full body contact activities  □ Canoe/Kayak launch
□ Wading area  □ Boating  □ Pet swimming/drinking area
□ Fishing  □ Water Fowl Hunting  □ Water Supply
□ Not likely to come in contact with people or pets

Were pictures included (close-up and landscape): ________________
Estimated size of bloom:_____________________
Were Samples Collected (if yes, type and number of samples): ________________
Date HAB confirmed (How):  □ Visual  □ ABRAXIS Test Kit_______  □ ELISA_______

Priority Level Description: Priority water resources identified for HAB monitoring and response activities are prioritized based on the likelihood of public and/or pet contact and/or ingestion. A water resource manager identifies the priority level of a specific location based on local use, season, or special events. Priority levels may change.

Priority I – High risk of public and/or pet contact – public beaches, canoe/kayak launches, popular wading areas, designated waterfowl hunting sites, pet training and exercise areas, drinking water intakes, etc.

Priority II – Intermediate risk of public and/or pet contact – public marinas, docks, open water likely to be used for recreational activities with direct water contact

Priority III – Areas of public waters that represent minimized contact/ingestion risk - public fishing access locations and shoreline walkways with public water access
Appendix F: Information Notice

IF IN DOUBT, STAY OUT!

Have fun on and in the water, but know that blue-green algae blooms are a global problem in lakes, rivers and other water bodies. Their toxins may be too. Knowing how to identify harmful algae blooms (HABs) and being alert can keep you, your family and your pets safe!

Avoid contact with water that:
• Looks like spilled paint
• Has surface scum, mats or films
• Is discolored or has colored streaks
• Has green globs floating below the surface

And ALWAYS AVOID...

swallowing water from lakes or other water bodies!

To report a suspicious algae bloom contact the PADEP at 814-332-6839
For more information, visit www.paseagrant.org
Appendix G: Recreational Use Notice

TOXIC ALGAE ALERT

A suspected harmful algal bloom has made this location potentially dangerous for humans and animals

AVOID ALL CONTACT WITH THIS WATER and SURFACE SCUM

For more information contact the Pennsylvania Department of Environmental Protection (PADEP) at 814-332-6839
OR
Visit www.paseagrant.org
Appendix H: Avoid Contact Notice

HARMFUL ALGAE
AVOID ALL CONTACT WITH THIS WATER AND SURFACE SCUM

For more information contact the Park office or go to: www.seagrant.psu.edu
Appendix I: Dog Use Notice

HARMFUL ALGAE

TOXINS MAY BE PRESENT AT LEVELS HARMFUL TO DOGS
KEEP PETS OUT OF WATER AND AWAY FROM SURFACE SCUM

For more information contact the Park Office or go to: www.seagrant.psu.edu