

These questions were generated during outreach sessions conducted by the Division of Drinking and Ground Waters and the Division of Environmental Services between October 2015 and May 2016. Topics addressed in this document are:

- Terminology
- Cyanotoxins
- Sampling
- Year-Round Weekly Monitoring Requirements
- Increased Monitoring Requirements
- Purchased Water Systems
- Cyanobacteria Screening Samples
- Laboratories
- Sample Management

- Quantitative Polymerase Chain Reaction (qPCR) Analysis
- Other Analytical Methods
- Cyanotoxin Research
- Treatment Optimization and Cyanotoxin General Plans
- Public Notices
- Data Reporting
- Grant

Terminology

- Q: What is the difference between the action level and detection level (or reporting limit) for total microcystins?
- A: The action level for total microcystins is $0.3~\mu g/L$. When considering rounding and significant digits, this establishes an actual exceedance of the action level at $\geq 0.345~\mu g/L$. The detection level (or reporting limit) for total microcystins using the Ohio EPA method is currently $0.30~\mu g/L$. This leaves a very small window of detection that is not considered an action level exceedance (from $0.30~to~0.344~\mu g/L$). However, future refinements to the analytical method could result in a lower reporting limit.
- **Q:** Does "Genomic Cyanobacteria Screening" refer to qPCR?
- A: Yes

Cyanotoxins

- **Q:** How many other toxins are there?
- **A:** There are many cyanotoxins. The rules specifically address total microcystins. However, saxitoxins, cylindrospermopsin and anatoxin-a are also included in the Public Water System Harmful Algal Bloom Response Strategy (updated in 2016).
- **Q:** It has been suggested that the toxicity of a strain of algae is wholly attributed to the presence of the coding gene. Is there any indication that cellular regulation of gene expression—for example, transcription, translation, post-translational modification—are of equal or greater significance in the presence/absence of the gene in determining the toxicity of algal blooms?
- **A:** Yes, there are some methods that evaluate gene transcription, but there are a lot of different environmental triggers that turn the gene on and off. Of the methods that are available right now, the best approach is to evaluate the presence of the cyanotoxin-production gene. It is possible to have a gene present without cyanotoxin production; however, if cyanotoxins are being produced, the gene must be present. Therefore, we believe the cyanobacteria qPCR assay offers an effective tool to screen for potential cyanotoxin production.

Sampling

- **Q:** Will DES sample and analyze a public water system's weekly sample if, for some reason, they are unable to sample themselves?
- A: In general, public water systems are responsible for sampling and analysis for total microcystins, and also for collecting and shipping the cyanobacteria screening samples to DES. However, DES may be able to provide analytical services for a fee in some situations. There are many Ohio EPA accepted commercial and municipal laboratories offering sample analysis for public water systems. The list of accepted labs can be found here: epa.ohio.gov/Portals/28/documents/labcert/TotalMicrocystins.pdf.
- **Q**: Is there a requirement for reanalysis of a sample following a finished water detection?
- **A:** No. The reanalysis requirement appeared in an early draft of the rules, but was not included in the final rules.
- **Q:** Should the finished water sample be timed to match flow and represent the same water that was collected for the raw sample?
- **A:** No. The cyanobacteria screening raw water sample and the total microcystins raw water sample must be collected at the same time, but the finished water total microcystins sample only needs to be collected on the same day.
- Q: Does a system need to conduct raw water monitoring at all of its potential sources of water?
- **A:** No. For HAB rule compliance, surface water systems are required to collect a true raw sample from the source entering the treatment plant. The requirement is one sample per surface water treatment plant. Additional monitoring may be conducted voluntarily by the water system for consideration in treatment optimization and reservoir management.
- **Q:** If you have to recollect the qPCR (cyanobacteria screening) sample for whatever reason, would you then you have to recollect for total microcystins analysis too? Will a problem with the qPCR sample invalidate the corresponding total microcystins analysis sample?
- A: The bi-weekly qPCR cyanobacteria screening sample must be collected at the same time as the weekly total microcystins sample. Invalidation of the qPCR sample will not mean invalidation of the total microcystins sample. If a qPCR sample must be recollected, then the recollection must occur at the same time as the next total microcystins sample on the following week, within the bi-weekly sampling period. Please contact your Ohio EPA district office HAB coordinator with notification of resampling efforts.
- **Q:** If a qPCR (cyanobacteria screening) sample analyzed by DES indicates a potential for production of toxins other than total microcystins, where will samples for saxitoxins, cylindrospermopsin, etc. be sent? Will these samples then go to DES too?
- A: If a cyanobacteria screening sample indicates a potential for cyanotoxin production (saxitoxin or cylindrospermopsin), Ohio EPA will perform additional sample collection and follow up analysis by ELISA at our DES lab.
- **Q:** Where should the retested sample (Resample) be pulled from, the previously tested sample or a newly collected and processed sample?
- A: The resample should be a newly collected sample. The reanalysis requirement was not included in the effective rules. If an action level exceedance occurs, new resample and repeat samples must be collected from each raw water sampling point (LT2001) and each finished water sampling point (EP001), please see OAC Rule 3745-90-03 (A)(4) and (C)(2) for details.
- **Q:** How soon after June 1 does the sampling begin?
- **A:** The rules will go into effect on June 1, 2016. The first sampling date will be June 5, 2016 with weekly sampling periods defined as Sunday to Saturday.

- Q: June 1 is a Wednesday. If we were planning to do our sampling every Tuesday, do we wait until the following week?
- **A:** The rules are effective June 1. The first sampling time period will begin June 5 and from there, a week is counted from Sunday to Saturday, so any time within that timeframe (*i.e., Sunday, June 5 to Saturday, June 11 for the first sampling event*).
- **Q:** Are systems required to collect finished water samples during the November to April reduced monitoring period?
- **A:** No. Surface water systems that qualify for reduced seasonal monitoring will only be required to collect raw samples for total microcystins bi-weekly with the cyanobacteria screening sample. If microcystins are detected in the raw water during that time period, that will trigger finished water monitoring.
- **Q:** At what point in November will systems shift to the reduced monitoring schedule?
- **A:** Reduced monitoring schedules will be effective at the start of November and monitoring will begin the first or second week in November to coincide with the bi-weekly cyanobacteria screening sample.
- **Q:** Where exactly do we sample the raw source—in the source or at a pump station where it is at the first step being brought into our system but before any treatment has started?
- A: As a general rule you can use your LT-2 Surface Water Treatment Rule sampling location; however, if that sampling point is after a chemical addition, you have to temporarily turn off your chemical feeds, as you do in your LT-2 sampling, select an alternate location that would be more representative of a true raw sampling point, or in some situations Ohio EPA may approve the collection of "raw" samples that contain permanganate. You should contact your Ohio EPA district office HAB coordinator to help evaluate your options.

Year-Round Weekly Monitoring Requirements

- **Q:** Will systems considered ground water under the influence of surface water need to meet HAB monitoring requirements?
- **A:** Yes. "Ground water under the influence systems" are surface water systems and subject to all rules a surface water system is required to meet.
- **Q:** Can previous monitoring results be used to reduce the 12-month monitoring requirements?
- **A:** Yes, historic data can be considered by Ohio EPA when revising monitoring schedules. However, Ohio EPA intends to wait until one full year of data has been collected prior to reducing monitoring requirements.
- Q: Can other information be used also, such as MPA data or algal toxin analyses done with HPLC or LC MS/MS?
- A: For total microcystins compliance monitoring the rules specify the use of the "Ohio EPA Total (Extracellular and Intracellular) Microcystins—ADDA by ELISA Analytical Methodology, Version 2.2 (November 2015)" (Ohio EPA DES 701.0) method as the approved analytical method. However, the rules allow for the consideration of "other information provided by the public water system including data from other screening tools (such as phycocyanin sensors or phytoplankton enumeration) and treatment information" [OAC 3745-90-03 (A)(3)] to determine reductions in future monitoring schedules.
- **Q:** Which method is required for the weekly screening—ELISA or quantitative Polymerase Chain Reaction (qPCR)?
- **A:** The rules require routine <u>bi-weekly</u> cyanobacteria screening (qPCR) and routine <u>weekly</u> total microcystins monitoring (Ohio EPA DES 701.0). Ohio EPA's Division of Environmental Services (DES) will be conducting the qPCR analysis for at least the first year. Public water systems are required to submit samples for analysis of total microcystins to an Ohio EPA approved or certified laboratory.
- **Q:** Will the sampling requirements be on the monitoring schedule for 2016 for each system?
- **A:** Revised monitoring schedules reflecting the new Harmful Algal Blooms (HAB) monitoring requirements were mailed on Friday, May 6, 2016 to all Public Water Systems (PWS) with HAB monitoring requirements.

- **Q:** Have you looked at the cost to the public water systems for the 52 weeks of sampling? Is this not a hardship to smaller public water systems?
- A: Yes, as a requirement of the rule adoption process, we've considered the costs of the draft rules on public water systems, accounting for 52 weeks of sampling. Ohio EPA recognizes that this will come with a cost to public water systems to complete the required sampling and analysis. Ohio EPA made \$1 million dollars in grants available to public water systems to establish their own analytical capabilities to provide some assistance and relief for source water monitoring costs.
- **Q:** Could you sample late in the week the first week then early the next and run both sets of samples in one run the second week?
- **A:** Yes, as long as the samples are analyzed within five days. However, a public water system that does this will lose some of the flexibility in the 10-day advisory; if those samples were over an action level, then the water system has used up some of the time to adjust treatment.
- **Q:** If a public water system misses a week in the 52-week testing period will they still be able to get a reduction in testing the following years?
- **A:** Failure to submit samples would be a violation of the monitoring requirements. The circumstances surrounding the lack of a sample or samples would be considered as part of making a determination on reduced monitoring requirements.
- Q: If a surface water system only operates for a few weeks per year (or not at all), how will sampling be handled?
- **A:** They will need to sample during the time period they are open and operating. This is addressed in the opening statement for rule 3745-90-03.
- **Q:** How many screening samples are required and when is the first sample due?
- A: The rules require bi-weekly raw water cyanobacteria screening using qPCR and weekly raw and finished water routine microcystins monitoring, beginning June 5, 2016. Revised monitoring schedules reflecting the new HAB monitoring requirements were mailed on May 6, 2016, to all PWS with HAB monitoring requirements. The rules do not state a minimum number of samples which must be collected.
- **Q:** Is Ohio EPA specifying a day of the week for total microcystins or cyanobacteria screening sampling?
- A: No. The water system will be permitted to collect samples anytime within the seven-day week (Sunday to Saturday starting June 5, 2016). It is important to remember that total microcystins sampling must be collected at the same time as the cyanobacteria screening sample. If the water system intends to ship the cyanobacteria screening sample from one of the shipping hubs, there may be specific day requirements to ship, limiting the flexibility of total microcystins sampling.
- **Q:** If you have multiple sources and there are detections on a source you are not currently using, does that prevent you from going to reduced monitoring in the off-season?
- A: No.
- **Q:** Will reduced monitoring be automatic or do we need to apply for/request it?
- **A:** It will be automatic.

- **Q:** When we do daily sampling and only analyze once per week, we do not meet the five-day requirement. What do we do?
- A: All compliance samples must meet the five-day analysis timeframe required in the rule. Samples not specifically required for compliance must be analyzed in accordance with the method, which allows freezing to extend the hold time. If daily compliance sampling is required due a finished water detection you will need to analyze samples more frequently (within 24 hours of collection) or utilize a commercial lab that offers analysis within the required timeframe.
- **Q:** What if one of my batched samples that was not required for compliance has a detection?
- A: If it is a finished water sample you should contact your Ohio EPA district representative right away and collect a follow up sample to determine if your treatment is effective and if cyanotoxins are still present in your finished water. This will maximize the amount of time for treatment optimization and potentially avoid an action level exceedance in future compliance samples. While a finished water detection in a non-compliance sample would not automatically trigger daily sampling including resample and repeat samples, we would consider it when determining whether or not to issue a public notice prior to a repeat sample action level exceedance. A raw water detection in non-compliance samples would not trigger treatment technique requirements or increased monitoring.

Increased Monitoring Requirements

- **Q:** How was the 5 μ g/L in raw water concentration chosen as trigger for increased monitoring?
- A: $5 \mu g/L$ is the concentration where we typically see the exponential growth phase of a bloom and a rapid increase in microcystins concentration. Additionally, finished water detections at Ohio PWS have historically occurred when raw water levels exceed $5 \mu g/L$.
- Q: Will any consideration be given for reducing the "increased monitoring due to raw water microcystins concentrations above 5 μ g/L" for public water systems that have demonstrated the capability to remove microcystins or ways to avoid using raw water containing microcystins?
- A: Yes, there is a provision in the rules that allows public water systems to demonstrate treatment effectiveness in their Cyanotoxin General Plan and reduce monitoring to an alternative frequency. If the concentration is greater than the 5 µg/L trigger, the system would be required to submit a Cyanotoxin General Plan. It would be acceptable to submit and implement a Cyanotoxin General Plan before it is officially required under the rule in order to take advantage of this reduction in increased monitoring.
- **Q:** Following finished water detection, what is the significance of increased raw water monitoring?
- **A:** Increased raw water monitoring frequency following a finished water detection is very helpful to understand the current levels of cyanotoxins entering the plant and guide treatment adjustments by determining the levels of intracellular and extracellular toxins. It is also valuable to understand the changing levels of microcystins in the raw water (steady, increase, decrease) and correlate with levels in the finished water.
- **Q:** For raw detections between 0.3 and 5 μ g /L, do we have to do any additional monitoring?
- **A:** No, as long as the cyanotoxins are not detected in the finished water.
- **Q:** Are systems expected to collect three samples in the week a raw water detection triggers increased raw water monitoring?
- **A:** No. The increased monitoring requirement to three times per week will begin the week following the raw water detection.
- **Q:** If a system is conducting increased raw water monitoring can it take all three samples on the same day?
- **A:** No. The samples must be collected at least 24 hours apart.

Purchased Water Systems

- **Q:** Are systems purchasing water from an in-state surface water system required to perform compliance sampling between November and April?
- **A:** No. Consecutive water systems purchasing from in-state suppliers are not required to conduct routine microcystins or cyanobacteria screening sampling.
- **Q:** Is an in-state surface water system required to notify systems purchasing from them of a raw water detection?
- **A:** No. Surface water systems selling water are only required to notify their consecutive systems when they have a finished water detection.
- **Q:** Where is the sampling point for a system purchasing water from an out-of-state surface water system?
- **A:** The ideal location is an entry point tap in Ohio. If this type of tap is not available, please contact your Ohio EPA district HAB coordinator to determine the best sampling location.
- Q: Do purchased water systems have to develop treatment protocols?
- A: No.

Cyanobacteria Screening Samples

- **Q:** If my public water system has made arrangements to ship from a shipping hub and I miss the sample drop-off deadline, what should I do?
- A: In this case, the public water system would need to ship the sample overnight on the same day as collection to Ohio EPA's Division of Environmental Services (DES) in a disposable cooler. You should contact DES at (614) 644-4243 to schedule the samples so the lab is prepared to receive your shipment.
- **Q:** If my public water system has made arrangements to ship from a shipping hub that ships on Wednesdays, can I collect my cyanobacteria screening sample early, like Monday?
- **A:** No. Cyanobacteria screening samples must be shipped on the same day as collection.
- **Q:** What type of delivery service will be used to ship samples from a hub to DES?
- **A:** Ohio EPA is negotiating contracts with currier services and commercial shipping services to find the best value for shipping samples.
- **Q:** Will systems have to pay for shipping from a hub?
- **A:** In 2016, Ohio EPA will cover the cost of shipping the cyanobacteria screening samples from Ohio EPA-approved shipping hubs.
- **Q**: What happens if I miss the two-week monitoring period for cyanobacteria screening and don't collect a sample?
- **A:** A Tier 3 monitoring violation will be issued with a requirement to public notice.
- **Q:** Who will conduct follow-up sampling if qPCR indicates the genes for cyanotoxin production are present?
- A: Ohio EPA will conduct all follow up monitoring for the cyanobacteria screening.
- **Q:** Does a system have to continue microcystins sampling if the gene for production of another cyanotoxin is present?
- A: Yes.
- **Q:** How soon after collection do qPCR samples need to be put on ice?
- **A:** Samples must be protected from sunlight and cooled on ice at 0-4°C immediately after collection and maintained at 0-4°C until analysis.

Laboratories

- **Q:** Does Ohio EPA intend to provide interim certification for laboratories that have the capabilities for qPCR methodology prior to 2017?
- **A:** No. Ohio EPA will be developing a standardized analytical methodology that it will use during the first year of analysis. Once the method has been developed, Ohio EPA will begin certifying laboratories for qPCR in June, 2017.
- Q: If new analysts are added will there be an additional certification fee?
- **A:** No. If a lab has paid the \$1,550 fee and obtained certification, a process is in place that allows a new analyst to be certified for total microcystins by submitting an acceptable MDL study and interim authorization application to the Ohio EPA Laboratory Certification Section.
- **Q**: Is the \$1,550 for certification fee over and above the \$1,800 we pay for microbiology certification?
- **A:** Yes. The laboratory certification fee for cyanobacteria screening and total microcystins will be a separate \$1,550 fee and cover certification for a three-year period.
- **Q:** Can you say something more about the auto-analyzer?
- A: The auto-analyzer allows for the ELISA method to be nearly fully-automated. All calibration standards, QC standards and samples are added to the 96-well plate by the instrument. The instrument is then able to add all the required reagents and perform all the wash steps. Once the required steps are completed, the instrument will then read the 96-well plate. There are a variety of auto-analyzers on the market. Please contact a member of the Laboratory Certification Section at <code>DWLabCert@epa.ohio.gov</code> or (614) 644-4245 to determine which auto-analyzer is acceptable.
- **Q:** Are there concerns with capacity of labs to handle routine total microcystins monitoring and/or increased monitoring during a HAB event?
- **A:** Ohio EPA is confident that the number of laboratories accepted in 2016 for total microcystins analysis is sufficient for meeting the rule requirements for monitoring. In the event that multiple public water systems are experiencing finished water detections simultaneously, Ohio EPA's Division of Environmental Services may be available for handling additional total microcystins analyses.
- **Q:** Can sample bottles be recycled? If so, what are the analytical requirements?
- **A:** The 100 mL total microcystins sample bottles and 40 mL VOA vials may be cleaned and reused. However, 5 percent of all cleaned sample bottles must be tested prior to reuse. At this time qPCR sample bottles may not be reused.
- **Q:** During the lab certification process and setting up the lab space, what is the recommended setup area for ELISA analysis? Can it be housed inside a microbiological laboratory?
- A: We have not really set specific requirements on the size of the space. You will need enough space to have all the equipment: reader, computer, refrigerator, freezer (you can use a water bath to help with the freeze-thaw), pH meters, etc. It can be housed in a microbiological laboratory or chemistry laboratory that has been reviewed by the laboratory certification group, and as long as there is sufficient space. One of the most important considerations for setting up the laboratory is to assure that a constant temperature can be maintained. Temperature is a significant contributor to the accuracy of your results.
- **Q:** Will labs be held accountable for failure to report?
- A: Yes.
- **Q:** Who will face the consequences if a lab does not meet the reporting deadlines?
- **A:** Depending on the situation, both the public water system and the laboratory are eligible to receive a violation if reporting deadlines are not met.

Sample Management

- **Q**: Does the requirement to test for microcystin within five days of collection apply if the samples have been frozen?
- **A:** Yes. The five-day time period to conduct analysis for compliance samples is related to the need for timely data, and is not based on preservation or hold-time considerations. Samples not collected for compliance purposes may be frozen to increase holding time beyond five days,
- **Q:** After collection of a sample for total microcystins analysis, how long does the lab have to complete analysis? How long do they have to report the result? In other words, how much time do they have to run the ELISA?
- A: Routine total microcystins monitoring samples must be analyzed within five days of collection. Results must be reported by the 10^{th} day following the month in which a sample is collected. The following samples must be reported by the end of the next business day after analysis is completed: all detections of microcystins in finished water samples; all results of microcystins samples collected in response to an exceedance of the microcystin action level in paragraph (A)(1) or (A)(2) of rule 3745-90-02 of the OAC; and all results for total microcystins in raw samples above 5 μ g/L. All results must be reported to both the Ohio EPA via eDWR and to the PWS.
- **Q:** What happens to the PWS if there is a shipping problem/error for the qPCR sample (cyanobacteria screening)? Will they get an NOV if a shipment problem causes delay, although it was still actually collected on time?
- **A:** If an error in shipping occurs during a PWSs qPCR collection week, the PWS should recollect the qPCR sample in tandem with the total microcystins sample during the next week of collection. If no sample is collected and analyzed during the two-week timeframe, then an NOV will be issued.
- **Q:** Some of the audience believes that a cyanobacteria presence will always be there. The "atmosphere" of the lab, cooler, etc. will be contaminated. How will Ohio EPA address contamination concerns with collective shipping and collective analysis because a "presence will always be there?"
- **A:** To ensure no contamination occurs at any point during sample preparation or analysis, Ohio EPA's Division of Environmental Services will be using certified sterile consumables and aseptic technique. The absence of contamination will be demonstrated through the use of blanks and a No Template Control (NTC) well. In regards to contamination from the cooler, the PETG bottle should already be closed when placed into the cooler, an effective avoidance of contamination. Additionally, bottles are wiped down as they come into the Sample Receiving areas at the lab providing another cross-contamination barrier.
- **Q:** What is the "holding" time for qPCR samples? How much shipping time is there for the samples to be received by DES after the time of collection?
- **A:** qPCR samples must be filtered within 48 hours of collection. Ohio EPA's Division of Environmental Services must receive samples as soon as possible, preferably within 24 hours of collection (no less than 36 hours), to allow sufficient time for sample processing.
- **Q:** What is the chain of custody for qPCR samples?
- **A:** The chain of custody documents the movement of the sample from collection to the laboratory. We have developed a joint sample submission form and chain-of-custody form that will be used when you submit the qPCR samples for analysis. This form and sample collection procedures were included with your Revised Monitoring schedules mailed on May 6, 2016.
- **Q:** The SOP says you can freeze the samples to extend hold times but we have also been told that this cannot be done. Which is correct?
- **A:** All compliance samples must meet the five-day analysis timeframe required in the rule. Compliance samples can be frozen prior to analysis, but they must be analyzed within five days of collection (all samples must go through a minimum of three freeze/thaw cycles prior to analysis, according to the method). Hold times can be extended beyond five days for samples not specifically required for compliance, as long as they are frozen within five days of collection.

Quantitative Polymerase Chain Reaction (qPCR) Analysis

- **Q:** Is qPCR an approved U.S. EPA or Standard Method test?
- **A:** qPCR is not currently an approved U.S. EPA method. Ohio EPA's Division of Environmental Services intends to develop standardized analytical methodology and begin certifying laboratories beginning June 1, 2017. Until then, DES will analyze all the qPCR samples required under these rules.
- **Q:** Is there a draft Standard Operating Procedure for qPCR methodology?
- A: DES will complete research and intends to develop a standardized qPCR analytical method including Quality Control (QC) requirements for certification by Ohio EPA beginning June 1, 2017. The Quality Control criteria will be established to enable laboratory certification staff to certify other laboratories for cyanobacteria screening by qPCR. This standard method will be developed in collaboration with state and national experts in qPCR.
- **Q:** Will qPCR be the only acceptable method for the screening requirement or can a utility perform traditional algal counts and/or identification to meet the screening requirement?
- A: At this time the only acceptable cyanobacteria screening method is a cyanobacteria qPCR assay. Many traditional cyanobacteria screening tools cannot be certified by Ohio EPA due to the limitations in reproducibility and comparability among equipment and methods. Ohio EPA will continue to evaluate and consider additional screening methods that can be certified.
- **Q:** Can a utility perform qPCR on its own to meet the requirement?
- **A:** Not at this time. Until Ohio EPA has a standardized analytical methodology and provides certification for qPCR, all cyanobacteria screening will be done by Ohio EPA's Division of Environmental Services. Once a final method is available after June 1, 2017, a utility could become certified.
- **Q:** Does qPCR give you a toxin result?
- A: No, qPCR does not give a cyanotoxin concentration; it quantifies cyanotoxin-production genes present.
- **Q:** How will a public water system know if there is potential for saxitoxins, anatoxin-a, or cylindrospermopsin production by a cyanobacteria?
- **A:** qPCR will detect the presence of the cyanotoxin-producing genes. All surface water public water systems will be required to collect bi-weekly samples and ship to DES for qPCR analysis. qPCR results will trigger additional sampling and analysis by Ohio EPA. qPCR results will be directly accessible by the PWS through the eDWR system.
- **Q:** What follow-up requirements to the qPCR results will the water system be responsible for?
- **A:** All follow-up sampling related to the results of the qPCR analysis for cyanobacteria screening will be conducted by Ohio EPA. The water system will be responsible for follow-up sampling related to total microcystins detections, but not the cyanobacteria screening. The public water system may be asked to assist with sampling.
- **Q:** What will the cost per sample be for cyanobacteria screening and who will incur the costs?
- A: The initial cost estimates are between \$70 and \$140 per sample, but these costs will be refined as part of the process to develop a standardized analytical method for qPCR. Ohio EPA will not be charging public water systems for qPCR analysis conducted by DES. Public water systems will incur the cost for sample collection, analysis for microcystins and for shipping the qPCR samples to Ohio EPA's Division of Environmental Services. Shipping hubs have been created to coordinate the shipping and reduce associated costs.

Other Analytical Methods

- **Q:** What are the plans to utilize the new U.S. EPA analytical methods 544 and 545? It appears that the focus is totally on ELISA.
- **A:** The rules only allow for use of the Ohio EPA DES 701.0 method. The U.S. EPA health advisory levels are based on total microcystins, including all of the microcystins variants/congeners. The 544 method does not provide data for total microcystins. The rules do indicate the Director could authorize additional methods as more information becomes available in the future.
- **Q:** Is there an SOP for cylindrospermopsin and saxitoxins? Who will be doing the testing?
- A: DES has developed an ELISA SOP for both cylindrospermopsin and saxitoxins. LC-MS methods have also been established for cylindrospermopsin, saxitoxins and anatoxin-a. Ohio EPA will collect and analyze samples for cylindrospermopsin and saxitoxins (using ELISA SOPs) and anatoxin-a (LC-MS method) if cyanobacteria screening data indicate those cyanotoxins may be present. Ohio EPA will conduct additional analysis using a LC-MS method following any finished water cylindrospermopsin, saxitoxin or anatoxin-a detections.

Cyanotoxin Research

- **Q:** Have Ohio EPA cyanotoxin research studies been published or made available online? For example, occurrence studies that indicate microcystin variants other than LR are present?
- A: Yes. An article titled "Using the MMPB technique (2-methyl-3-methoxy-4-phenylbutyric acid) to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS)" was published in the journal Toxicon (104 (2015) 91e101). The authors, Amanda Foss and Mark Aubel with Greenwater labs analyzed Ohio samples as part of a microcystin variant analysis. The data or the full article can be provided by Ohio EPA upon request.

Treatment Optimization and Cyanotoxin General Plans

- **Q:** When will the treatment optimization guidance/template be finalized and will it be available on Ohio EPA's webpage?
- **A:** A final guidance was made available on May 20, 2016 and was posted on our webpage. PWSs were notified by email of the document's availability.
- Q: There are public water systems that are not at risk that have not had to submit a Cyanotoxin General Plan. Should they submit a Cyanotoxin General Plan to qualify for reduced monitoring with regards to the 5 μ g/L trigger for increased monitoring?
- **A:** A public water system can submit and implement a Cyanotoxin General Plan to demonstrate that the water system can provide reliable treatment to avoid detections and support a reduced monitoring frequency.
- **Q:** What triggers the Cyanotoxin General Plan?
- A: Any detection of microcystins in finished water or microcystins in raw water at the intake above 1.6 μ g/L (two detections within a calendar year) (Rule 3754-90-05(B)). This will only apply if the samples are collected after the effective date of the rule.
- **Q**: Will a Treatment Optimization Protocol be required if the raw water has detections of 1.6 μg/L microcystins? Why?
- A: The rules would require a public water system (not including consecutive systems) to develop and submit written treatment protocols following any detection in raw or finished water, not just for concentrations at or above 1.6 µg/L. The purpose of a treatment protocol is to ensure the public water system has investigated the capability of its existing treatment plant to remove microcystins and outlined procedures for optimization to prevent a breakthrough of microcystins into the treated finished water. Most surface water systems have the ability to make short-term treatment adjustments to effectively target microcystins removal during a HAB event, but these adjustments are not necessarily consistent with normal operational needs. The treatment protocols must address treatment adjustments to be made under various raw and finished water conditions. In developing the protocols, the public water system must review and optimize existing treatment for microcystins removal, utilizing techniques that avoid lysing

cyanobacterial cells, optimize removal of intact cells, optimize barriers for extracellular cyanotoxin removal or destruction, optimize sludge removal and discontinue or minimize backwash recycling.

- **Q:** Will guidance be available to satellite (consecutive) public water systems for the locations and number of samples to be taken if the system they purchase from exceeds an action level?
- A: Guidance on distribution system sampling for cyanotoxins has been developed as is available on our webpage. Ohio Administrative Code rule 3745-85-01 requires each community water system to develop contingency plans for supplying water to the public in the event of an emergency. Satellite systems which purchase treated surface water will need to include a HAB response and recovery procedure in their contingency plans. This includes the need for distribution sampling for cyanotoxins. The goal of distribution sampling is to potentially isolate portions of the distribution system and limit the extent of an advisory. The sampling points for cyanotoxin sampling may coincide with existing sampling points for total coliform. Public water systems need to ensure the sampling points are accessible at any hour and day of the week. These points need to include interconnections with other public water systems, input and output from finished water storage and areas of the distribution systems served by different sources. This information is included in the Public Water System Harmful Algal Bloom Response Strategy (updated in 2016). In addition, characterization of the distribution system should consider areas that can be isolated by valving, areas that achieve a minimum contact time and if booster chlorination is used, pressure zones and how they are fed.
- Q: Our system regularly exceeds the 5 μ g/L in the raw water. Will we be able to get approval to increase the raw water trigger to 10 μ g/L or more?
- **A:** You could make the request with your Cyanotoxin General Plan and would have to provide documentation and rationale to demonstrate that your current treatment is effective with high levels of cyanotoxins in your raw water and that you have a good understanding of the range and type of cyanotoxins potentially present in your source water.
- **Q:** If a general plan makes the case that the existing practices are sufficient to prevent exceedances of the microcystins action levels, must this submittal still be prepared by a PE? In this case, the general plan is likely to involve less than \$5,000 in cost and the plans will not include additional treatment for removal, inactivation or chemical treatment of a health-based contaminant.
- **A:** The general plan will be required to be stamped by a PE, regardless of whether you are using existing infrastructure or recommending additional treatment, to insure compliance with a health-based standard (3745-91-03(B)(2)).
- **Q:** What concentrations of toxins do we optimize for? We've only seen 0.6 ppb, but are we supposed to plan to optimize for the highest that has been seen in Lake Erie, >50 ppb? We don't have the data necessary to really create an optimization plan. We're doing bench studies with purchased toxins. This is expensive and time-consuming. Small systems and systems that only have 30 days to prepare their protocol may not be able to do this.
- A: We are trying to get more data to better understand the dynamics of the source waters. We funded microcystins testing and other monitoring equipment and may be making more grant money available. Ohio EPA may have additional information available to help you characterize source water conditions and identify the type and range of toxin levels potentially present in your source water. Ultimately, your source water conditions and treatment plant capabilities are unique. We are not expecting a major study in your optimization protocol, but you may want to plan to conduct a study as part of your protocol.
- **Q:** What do I need to submit to demonstrate that my existing treatment is effective? Is data showing raw detections but no finished water detections enough?
- **A:** No, you will need to submit more than just historic data. We are currently developing guidance and minimum requirements for making a demonstration of effective treatment for reduced monitoring in the General Plan.

- **Q:** A PE is required for the general plan but PEs don't know about HAB treatment. Operators of record are not required to be PEs.
- **A:** The general plan is supposed to be a more holistic look at the PWS' treatment that incorporates all treatment objectives. If you were having a problem meeting disinfection by-products (DBPs), for example, your general plan would propose a solution that allows you to meet the HAB requirements and the DBP requirements. Infrastructure improvements may be necessary, which would require submittal of detailed plans.
- **Q:** We are being penalized by being proactive and conducting historic voluntary sampling? (Meaning that the optimization protocol is triggered upon effective date with 2015 data)
- **A:** We appreciate those who sampled proactively and do not view the initial treatment optimization protocol requirement as a punishment. Those systems that had raw or finished water detections last year are more at risk for finished water detection this year and in the future. It is beneficial and protective to have a treatment optimization protocol as soon as possible so you can be prepared for the 2016 HAB season. If you already have effective treatment, you can document that in your protocol. The objective of the requirement is to prevent finished water detections and avoid issuing advisories.
- **Q:** Will a system's treatment protocols be available to the public?
- A: No.
- **Q:** Will the HABs contingency plan developed last year be applicable to the treatment protocols?
- **A:** Yes. Depending on the details related to treatment in the contingency plan, it may be used as a starting point for development of the treatment optimization protocols.

Public Notices

- **Q:** Are public notices delayed until there are 10 days above the action level?
- A: No, the rules take an approach consistent with the Public Water System Harmful Algal Bloom Response Strategy. When U.S. EPA established the health advisory levels based on 10-day exposure, it encouraged states to treat them as acute standards with flexibility. The Strategy and draft rules provide for a resample of treated water and then a repeat sample. If the repeat sample is above a threshold an advisory public notice would be required at approximately 48 hours after the initial detection. The rules do provide for an extension of that time based upon specific bloom and public water system information.
- **Q:** Do the action level triggers for public notice include the $>5 \mu g/L$ in raw water?
- A: No. The action levels under the new rule only require public notification following exceedances in the finished water. Exceeding 5 μ g/L in raw water will initiate additional monitoring requirements and other treatment technique requirements.

Data Reporting

- **Q:** How will these results be reported by the 10th day eBiz?
- **A:** Yes, the certified laboratories will use Ohio EPA's eBusiness Center and Ohio EPA's eDWR reporting software to report the results.
- **Q:** Why was next day reporting of detections of cylindrospermopsin and saxitoxins mentioned if Ohio EPA will be conducting all analyses of the cyanobacteria screening samples?
- A: Next day reporting of cylindrospermopsin or saxitoxins gene detections was included in the rule because in the future, labs outside of Ohio EPA's Division of Environmental Services may be conducting these analyses and therefore subject to the reporting requirements. Timely reporting is necessary since it triggers follow-up sampling by Ohio EPA.

- **Q:** Is the public water system required to report total microcystins results on the monthly operating report?
- **A:** No. Total microcystins results will be reported electronically by the lab conducting analysis. This is similar to the way chemical monitoring requirements are reported.
- **Q:** How will the total microcystins and cyanobacteria screening data be reported to the media?
- **A:** Ohio EPA's HAB database will house the routine total microcystins data which will be accessible by the public and media. The results of the cyanobacteria screening and treatment train microcystin samples will be available upon request through the established public records request and file review process.
- **Q:** Are public water systems required to conduct additional source water monitoring to qualify for a reduced monitoring schedule in the future?
- **A:** No. But it would help build your case for reduced monitoring if you have additional data showing your ability to reduce and eliminate cyanotoxins in the finished drinking water and to characterize your source water conditions.
- **Q:** I have been sampling for HABs at multiple places each year. Do you still want that data even though the rules only require one raw sample per plant?
- **A:** Yes, please submit routine compliance results including raw, finished and distribution as well as any "special purpose" samples through eDWR and any other HAB monitoring data through the HAB mailbox just as you have in the past.
- Q: With respect to raw samples >5 μ g/L, is it sufficient to report >5 μ g/L or do they need to do dilutions to get a real result? How many dilutions would they need to do? What if there are two different answers off of two different dilutions, which result should be reported?
- A: Samples can be reported as >5 μ g/L, however, reanalysis with at least a 2x dilution is recommended to confirm the result is >5 μ g/L and will trigger increased monitoring. A quantified result obtained through dilution and reanalysis may also help with treatment optimization (for instance, adjust carbon feed based on actual raw water concentration). With regards to what result to report, if the first result is >5 μ g/L and the result after a 2x dilution is 4.6 μ g/L the lab would report the concentration that is within the calibration range (4.6 μ g/L). If, however, a dilution results in a non-detect value (because sample dilution was too great), the >5 μ g/L value should be reported, or the lab should select a smaller dilution factor and reanalyze. If a larger (10x or greater) dilution still results in a value greater than the calibration curve, the system can either report the value as >50 μ g/L (in the case of a 10x dilution) or do additional dilutions and reanalyze until a result is within the calibration range.
- **Q:** What are PWSs that run total microcystins more frequently than the rules supposed to do?
- **A:** The routine total microcystins must be paired with the bi-weekly qPCR sample. All other samples must be reported as special purpose.
- **Q**: If there is more than one source for raw water used by a facility, how should the sample be identified in eDWR?
- **A:** If more than one source for raw water is used at the same time by a facility, then the raw water compliance sample should represent the water that is fed to the treatment plant. It should be labeled with the SMP ID of LT200X and submitted through eDWR. If there are additional raw samples, they should be reported as "special purpose" samples.
- Q: What does EP001 and LT2001 mean?
- A: EP001 is the sampling location name given to the finished water sampling monitoring point at the entry point to distribution (first available tap following all treatment). LT2001 is the sampling location name given to the raw water sampling monitoring point currently used for LT-2 (cryptosporidium) raw water sampling.
- **Q**: Before the rule becomes effective, where are labs required to report the data (spreadsheet or eDWR)?
- A: Prior to June 1, 2016 report total microcystins samples results via eDWR and mark as Special Purpose (SP).

- **Q**: What is the rule reference for "all samples must be reported?"
- **A:** OAC 3745-90-04(D) Reporting of analytical data for determining compliance with this chapter shall be completed in accordance with rule 3745-89-08 of the Administrative Code.
- **Q:** How is qPCR data reported?
- **A:** In 2016 data will be electronically reported by our Division of Environmental Services through eDWR. Public water systems will not be required to report the qPCR results since it will be reported directly from the lab.
- **Q:** Will the qPCR results be shared with the utilities and how long will it take to get the results?
- **A:** Yes, the data will be shared; PWS may access their information via eDWR. Any additional monitoring triggered by the qPCR results would be conducted by Ohio EPA in 2016.

Grants

- **Q:** Will Ohio EPA offer grant assistance for third-party testing?
- **A:** No. We have no plans to offer grants for third-party testing.
- Q: Where can a public water system find grant money to purchase an auto-analyzer?
- A: We have an application for grant money available on Ohio EPA's website (*epa.ohio.gov/ddagw/HAB.aspx*). All HABs –related material is available on our HABs webpage and funding opportunities are highlighted on the "Funding" tab. While our initial \$1M has been allocated, we are planning to make additional grant funds available for cyanotoxin monitoring equipment starting on July 1, 2016. If you are interested in applying for these grant funds, we recommend that you complete an application and submit it as soon as possible to be on the list for the funding.
- **Q:** Can public water systems modify their grant applications to remove microscopes, since the microscopes will effectively become obsolete for HAB monitoring with the use of qPCR?
- **A:** We think microscopes are a beneficial tool for public water systems to have access to for algal identification. This information can be used to inform reservoir management decisions. If a water system would want to change its application and not proceed with the purchase of a microscope, they may do so.
- **Q:** Will Ohio EPA be reviewing sondes and fluorometers as other screening methods for HAB monitoring?
- A: Although data from sondes and fluorometers are not included in the rules due to the variability of the equipment and difficulty in developing a validation methodology, we think they are still an extremely valuable tool for public water systems. The data helps the system characterize and track source water conditions so staff can make rapid adjustments in treatment rather than wait until they have results of a weekly sample. We evaluated the use of phycocyanin concentrations as a screening tool, but unfortunately the concentrations are highly variable and dependent on the type of cyanobacteria present, turbidity and other environmental factors, so it was not useful as a quantitative screening tool.
- **Q:** We already sent in an application for HABs monitoring funding. Do we need to reapply, or are we good for the next funding available?
- **A:** No, you do not need to reapply unless you are changing the request (for instance, equipment type, costs, etc.). If you're going to add equipment or request for additional funds, you should send in the amended request.

Other Topics

- **Q:** Will the NOAA satellite be running this HAB season?
- A: Yes. The weekly bulletins for Lake Erie will continue and are available online at http://www2.nccos.noaa.gov/coast/lakeerie/bulletin/bulletin_current.pdf. Ohio EPA is currently working with NOAA to develop a similar tool for larger inland lakes and portions Ohio River.