Wyoming Department of Environmental Quality, Water Quality Division
Watershed Protection Program

HARMFUL CYANOBACTERIAL BLOOMS: CYANOTOXIN COLLECTION (LENTIC)

(**EFFECTIVE DATE:** APRIL 2021)

Introduction	Cyanobacteria can produce cyanotoxins that can affect the skin, liver and central nervous system in people, pets and livestock. Common cyanotoxins include microcystin, cylindrospermopsin, anatoxin-a and saxitoxin. The procedure for cyanotoxin sample collection varies per type of cyanotoxin but follows the general steps in the SOP for **Harmful Cyanobacterial Blooms: Cyanobacteria Collection (Lentic).** Specific instructions for cyanotoxin sample preparation, processing, and preservation are included below.
Quality Control	Samplers follow the SOP. Include field quality control samples during sample collection (see **Field Duplicates** and **Blanks** below).
Equipment	Hand-held open mouth sampler (optional) Plastic 30-60 mL amber PETG bottle (one for microcystin and cylindrospermopsin) Glass 40 mL amber vials (one for anatoxin-a, one for saxitoxin) Sample diluent (for anatoxin-a and saxitoxin preservation) Disposable, powderless gloves Elbow length or shoulder-length gloves (if submerging hands and arms) Goggles and mask to cover nose and mouth (if spray is unavoidable) Plastic knee boots, hip waders or chest waders (if collection requires wading) Cooler with dry ice (wet ice or ice packs if samples will be frozen within 24-36 hours) Digital camera to record appearance of bloom Pens and permanent markers Data sheet Packaging and packing tape Bags for shipping Laboratory forms (e.g., chain of custody)
Containers	Per request of analyzing laboratory. *NOTE*: Cyanotoxins are organic compounds that can adhere to certain sample containers, resulting in absorptive loss of toxin. Therefore, sample containers must be made of fluorocarbon polymers such as PETG, metals such as stainless steel, or glass.
Field Duplicates And Blanks	Include duplicates and field blanks in at least ten percent (10%) of all collected samples. Since cyanotoxin sampling is generally in response to complaints and therefore unpredictable, collect a duplicate and field blank with the first sample in each series (i.e., a duplicate and field blank for the 1st of 10 samples, another duplicate and field blank for the 11th of 20 samples). Duplicate sampling consists of one sampler using two bottles simultaneously. See SOP for **Blanks** for field blank information.
Preservative	Preservatives are only required for ambient anatoxin-a and saxitoxin samples as well as treated drinking water samples; ambient microcystin and cylindrospermopsin samples do not need to be

preserved. Ambient anatoxin-a and saxitoxin samples should be preserved with sample diluent (i.e., buffer solution) immediately following collection to prevent absorptive loss of toxin. Preserve samples to a 1:9 ratio (e.g., 2 mL of diluent per 18 mL of sample). Note that analytical results will be adjusted based on the dilution factor of the diluent.

Holding Time

Microcystin, cylindrospermopsin and saxitoxin samples can be stored on ice or refrigerated up to five (5) days while anatoxin-a samples can be stored on ice or refrigerated up to 28 days. Cyanotoxin samples must not exceed 10°C while being stored or shipped. If microcystin, cylindrospermopsin and saxitoxin samples will not be analyzed within five (5) days, or anatoxin-a samples within 28 days, samples should be frozen. Frozen cyanotoxin samples must be analyzed within 180 days.

NOTE: Freezing will cause cyanobacteria cells to lyse, hence frozen samples are only appropriate for total-concentration analysis (i.e., sum of intracellular and extracellular toxins). If freezing, ensure complete mixing of samples and sufficient room for thermal expansion within the sample container. To determine total cyanotoxin concentrations, laboratories generally do three (3) freeze-thaw cycles to lyse cells. Accordingly, it saves laboratory processing time to freeze samples immediately after collection by storing and shipping on dry ice. Check with analyzing laboratory to determine if samples should be immediately stored and shipped on dry ice.

Safety Precaution

Always wear disposable, powderless gloves when collecting, processing and preserving samples. If sampling requires reaching or submerged arms and hands, wear shoulder-length gloves when collecting cyanotoxin samples. Wear googles to prevent possible toxin exposure to the eyes, especially under windy conditions or when spray is unavoidable. Avoid inhalation of spray by wearing a mask. Use chest waders and personal floatation devices if wading offshore. Never ingest water or allow skin contact. Do not touch hands to mouth or other exposed areas of the body before washing. Wash hands with soap and water as well as rinse all equipment with water after collections.

Procedure

The procedure for collecting cyanotoxin samples for total concentration analysis is presented below. Outsourced laboratories may have specific protocols in place for sample processing, preservation, and shipping. Accordingly, the procedures presented here may be modified.

1. Review *Safety Precaution* for information on safety equipment and protocols to prevent exposure to cyanotoxins.

2. To determine potential health risks, collect samples at locations with the greatest potential for human and/or animal exposure to cyanotoxins. Depending on the location of the bloom, sampling locations may include beaches, shoreline access areas, boat ramps, docks, marinas, or open water. Be mindful of wind direction as cyanobacteria and therefore cyanotoxins may accumulate in downwind areas. Note sample location on data sheets and sample labels.

3. Wear disposable, powderless gloves during sample collection. Collections will generally be made from the shoreline or sampling from a dock or boat, depending on the location of the bloom. When cyanobacteria are concentrated in nearshore or littoral zones, target the

densest portion of the bloom that also represents the area of greatest exposure potential. Sample non-wadeable or open water from a boat or other reliable structures (e.g., dock) and target areas of greatest exposure potential.

4. When collecting a sample, use an open-mouth sampler or sample bottle. If a surface scum is present, hold the bottle parallel to the water surface and collect both scum material and surface water immediately below the scum (i.e., top 1-2 inches). For diffuse blooms or those with cyanobacteria distributed throughout the water column, invert and submerge the bottle to elbow depth. Once at elbow depth, revert the bottle and raise to the water surface such that the bottle samples the water column as evenly as possible. If using a sampler, draw off each sample into the sample bottle until desired volume is reached. Never fill a sample bottle more than one-half (1/2) full. An over-filled sample bottle may shatter during step six (6) and cannot be analyzed.

5. Wear disposable, powderless gloves when preserving and processing samples. Microcystin and cylindrospermopsin samples do not require any preservative. Preserve anatoxin-a and saxitoxin samples with sample diluent immediately after collection. The required amount of preservative for each anatoxin-a and saxitoxin sample are as follows:

    a. A ratio of 1:9 (2 mL of diluent per 18 mL of sample).

6. Store all samples out of light and on ice. Freeze microcystin, cylindrospermopsin and saxitoxin samples if they will not be shipped and analyzed within five (5) days and if anatoxin-a samples will not be shipped and analyzed within 28 days. Frozen cyanotoxin samples must be analyzed within 180 days. When freezing, ensure sufficient room for thermal expansion in the sample bottle. To determine total cyanotoxin concentrations, laboratories generally do three (3) freeze-thaw cycles to lyse cells. Accordingly, it saves laboratory processing time to freeze samples immediately after collection by storing and shipping on dry ice. Check with analyzing laboratory to determine if samples should be immediately stored and shipped on dry ice.

7. Label samples bottles according to laboratory guidelines. The following information is required (see SOP for **Sample Labeling):**

    a. Site name and/or location (e.g., beach, dock, boat ramp)
    b. Sample ID (Initials-YY-Julian Day-Sample No.)
    c. Date (mm-dd-yyyy) and time (24 hr)
    d. Preservative type and volume (mL) if applicable
    e. Number of freeze-thaw cycles (if frozen prior to sending to laboratory)
    f. Type of analysis (e.g., microcystin, saxitoxin, etc.)

8. Prior to shipping, contact analyzing laboratories to confirm shipping protocol and schedule. Typically, samples are packed in double bags, placed in coolers on wet or dry ice, and shipped overnight to arrive at the analyzing laboratory the next morning. Samples are not to be shipped on Fridays, Saturdays, or the day before a holiday as recipient laboratories will likely be closed. Include paperwork required by the recipient laboratory in all shipments.

Check all samples for correct labelling. Ensure that samples are on ice and in the dark during shipping.

9. Sample frequencies are determined on a case by case basis and depend on the overall objective of the study. If multiple samples are collected to monitor cyanotoxins over time, it is recommended that each sample be collected a minimum of 24 hours apart.

References

Abraxis Algal Toxins, Fresh Water (Cyanotoxins), Fresh Water Kits User Guides, accessible at https://www.abraxiskits.com/products/algal-toxins/

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US EPA, 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. US Environmental Protection Agency, Office of Water, Washington, DC.

US EPA, 2017. Detection of Algal Toxins in Surface Water Samples Using Abraxis Cyanotoxin Automated Assay System. Standard Operating Procedure BIOLM-008. US Environmental Protection Agency, Region 8 Laboratory, Golden, Colorado.

US Geological Survey, 2008. Cyanobacteria in Lakes and Reservoirs: Toxin and Taste-and-Odor Sampling Guidelines. Cyanobacteria, Version 1.0, Chapter A7, Biological Indicators.

Revision History

Date	Details of Revision	Revised by:
6/30/2017	New	M. Thomas
3/22/2018	Revisions to June 2017 version	M. Thomas
4/1/2021	Revisions to March 2018 version	M. Thomas