New Hampshire Department of Environmental Services
CyanoHAB Response Protocol for Public Water Supplies

7/30/2020

The following describes NHDES' protocol for responding to suspected blooms of harmful cyanobacteria (cyanoHABs) in public water supply sources. An overview of the protocol is provided in the attached flow chart. This protocol uses a tiered approach, with screening and monitoring steps leading to actions including optimizing treatment and notifying the public if test results indicate that cyanotoxins are or may be present at levels of concern.

This describes the approach for water systems that are **not** conducting monitoring or on-site analysis of cyanotoxins, cyanobacterial pigments, or other cyanobacteria-related water quality parameters. Water systems that are conducting such monitoring or analysis should develop a customized protocol in consultation with NHDES.

Contacting NHDES

Jody Connor Limnology Center (JCLC): Call in the following order
(603) 848-8094 (Cyanobacterial Bloom Hotline - primary contact number for Cyanobacteria issues; Amanda McQuaid)
(603) 271-0698 (Beach Coordinator office)
(603) 271-8865 (JCLC Director office)

Drinking Water and Groundwater Bureau
(603) 271-2513 (8:00 AM – 4:00 PM weekdays except holidays)
(603) 223-4381 (New Hampshire State Police - outside NHDES business hours)

Key terms used in the flow chart and the protocol:
BLOOM – source water where the suspected bloom appears to be at its worst
cyanoHAB – harmful cyanobacteria bloom
DWGB – NHDES Drinking Water and Groundwater Bureau
ELISA – enzyme-linked immunosorbent assay (Quantiplate) test for total microcystins (ADDA) (modified EPA method 546)
FINISHED – finished water entering the distribution system
JCLC - NHDES Jody Connor Limnology Center
LC/MS –Liquid Chromatography/Mass Spectrometry for analysis of specific microcystins, nodularin, anatoxin-a, and cylindrospermopsin (EPA Methods 544 and 545)
OPEN WATER – an area of the lake or reservoir between the visible BLOOM and the intake
PWS – public water system
RAW – raw water entering the treatment plant

1. **All PWSs using surface sources are advised to conduct daily visual surveillance** of their source water(s), at a minimum. NHDES recommends that PWSs using sources with a history of suspected cyanobacterial blooms implement monitoring programs that incorporate continuous or frequent monitoring of source water or raw water for phycocyanin, chlorophyll-a, pH, and other parameters, along with daily observations of weather conditions with a view to identifying conditions that are associated with the development of cyanoHABs. NHDES recommends that those water systems also participate in the Cyanobacteria Monitoring Program coordinated by USEPA's New England Regional Laboratory in Chelmsford, Massachusetts. Finally, NHDES recommends that PWSs with a history of suspected cyanoHABs have on hand sample bottles

prepared by NHDES Jody Connor Limnology Center (JCLC) or sample bottles prepared by another lab following their specific protocol.

2. **When there are visual signs of a bloom** or water quality parameters (e.g., pH, turbidity, taste/odor) indicate a suspected cyanoHAB, the PWS should contact NHDES - JCLC and the JCLC will notify DWGB of the confirmed bloom.

3. **Take samples of the bloom, open water, raw, and finished water in brown, 125 – 500 mL HDPE bottles supplied by NHDES JCLC**. Refer to the attached "Cyanobacteria and Cyanotoxin Sampling for Public Water Systems - Sample Collection, Preservation, Shipment, and Storage." (Whenever the PWS brings samples to the NHDES - JCLC for cyanotoxin analysis, they will be provided with another set of sample bottles.)

4. **JCLC will visually identify and determine the density of cyanobacteria in the samples.** Based on the cyanobacterial cell counts JCLC staff will consult with DWGB and determine whether toxin analyses should be done right away (or batched for later) and whether additional sampling should be done, either by the PWS or by NHDES. In most cases, DWGB staff will follow up with the PWS. If immediate action such as treatment adjustments is indicated, DWGB will follow up with the PWS. If toxigenic cyanobacteria are identified and in concentrations above 70,000 cells/mL, JCLC will make the appropriate decision as to which of the available toxin testing options seem(s) appropriate (See Tables 1 and 2 for cyanobacteria and associated toxins). If decisions are made to use LC/MS methods, JCLC will first consult with DWGB, and the PWS on the decision to run such tests and in what frequency.

   Toxin analyses may be done by the JCLC (ELISA for total microcystin) and/or one of the labs that can conduct additional toxin testing (LC/MS methods for specific cyanotoxins). Depending on the results, DWGB may ask the PWS to continue to sample water, will work with the PWS to optimize treatment, and will consider asking the PWS to **issue an advisory**. *Public notification templates are available.*

Attachments

- CyanoHAB Response Protocol flow chart
- Table 1: Common NH Cyanobacteria and Associated or Known Toxins
- Table 2: Cyanotoxins and common modes of action
- Table 3: Labs Conducting Toxin Testing using EPA Methods 544, 545, and 546
- Cyanobacteria and Cyanotoxin Sampling for Public Water Systems - Sample Collection, Preservation, Shipment, and Storage
- How to Make an Integrated Tube Sampler

For more information on cyanobacteria and what they might look like, refer to cyanos.org.

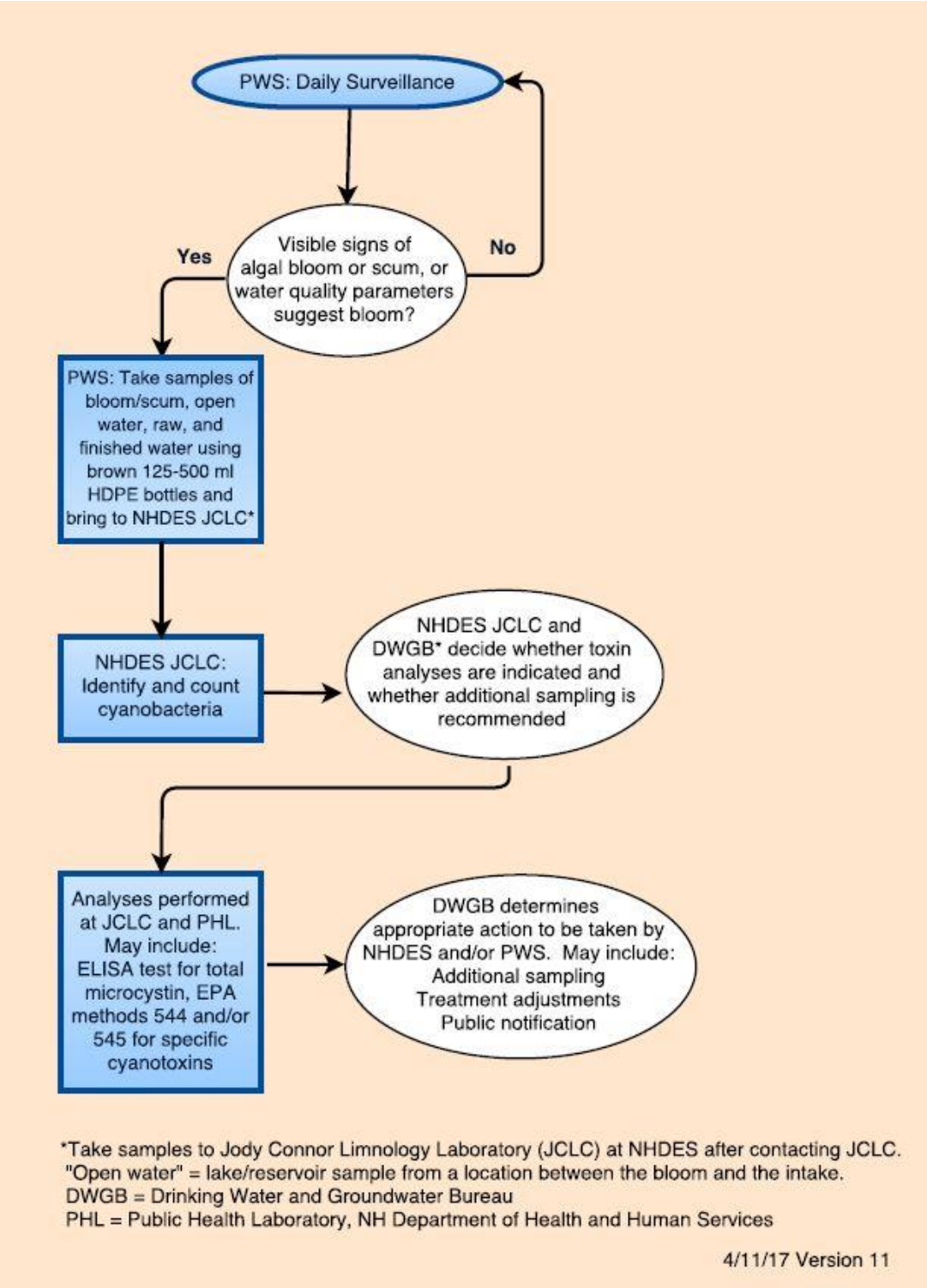
PWS: Daily Surveillance
Visible signs of algal bloom or scum, or water quality parameters suggest bloom?
Yes
No
PWS: Take samples of bloom/scum, open water, raw, and finished water using brown 125-500 ml HDPE bottles and bring to NHDES JCLC*
NHDES JCLC: Identify and count cyanobacteria
NHDES JCLC and DWGB* decide whether toxin analyses are indicated and whether additional sampling is recommended
Analyses performed at JCLC and PHL. May include: ELISA test for total microcystin, EPA methods 544 and/or 545 for specific cyanotoxins
DWGB determines appropriate action to be taken by NHDES and/or PWS. May include:
Additional sampling
Treatment adjustments
Public notification
*Take samples to Jody Connor Limnology Laboratory (JCLC) at NHDES after contacting JCLC.
"Open water" = lake/reservoir sample from a location between the bloom and the intake.
DWGB = Drinking Water and Groundwater Bureau
PHL = Public Health Laboratory, NH Department of Health and Human Services
4/11/17 Version 11

Table 1: Common New Hampshire Cyanobacteria and Associated or Known Toxins

The toxin test that NHDES may advise is first based on the presence of potentially toxic cyanobacteria, then on the concentration of cells per volume of water (currently 70,000 cells/mL), followed by determining the type of toxin it may produce. JCLC will test by ELISA for total Microcystins/Nodularins and Anatoxin-a and/or a lab listed on Table 3 can test by LC/MS methods to determine 7 variants of Microcystins, Nodularins, Anatoxin-a, and Cylindrospermopsin. This is not a complete list of the cyanobacteria or the cyanotoxins. Not all toxins can be tested by JCLC.

Common Cyanobacteria Genera of New Hampshire	Typical Form Observed	Associated or Known Toxins
Anabaena/Dolichospermum	Filaments	Microcystins, Anatoxin-a, Anatoxin-a (S), Saxitoxins, Cylindrospermopsin
Anabaenopsis	Filaments	Microcystins
Aphanizomenon	Rafts of Filaments	Anatoxin-a, Anatoxin-a (S), Saxitoxins, Possibly Microcystins
Aphanocapsa/Aphanothece	Colonies or Single Cells	Microcystins
Coelosphaerium	Colonies	Microcystins
Chroococcus/Gloeocapsa	Colonies	Possibly Microcystins
Gloeotrichia	Macroscopic Colonies	Microcystins
Lyngbya/Phormidium	Benthic Filaments	Microcystins, Lyngbyatoxins, Anatoxin-a
Merismopedia	Rafts of Colonies	Microcystins
Microcystis	Variations of Colonies	Microcystins, Anatoxin-a
Nostoc	Macroscopic Colonies	Microcystins, Nodularins
Oscillatoria/Planktothrix	Filaments	Microcystins, Cylindrospermopsin
Spirulina	Filaments	Microcystins
Synechococcus/Synechocystis	Single Cells, Rarely Colonial	Microcystins and Saxitoxins
Woronichinia	Dense Colonies	Microcystins

Note:

- Some genera grouped here have variations in their taxonomic name or are similar in morphology.
- Species may vary significantly. This is not a complete list of the cyanobacteria.
- More than one type of cyanobacteria and toxin may exist in a typical bloom.
- Microcystins are the most common cyanotoxin in New Hampshire and New England.
- Associated toxins are typical and not guaranteed as research evolves.
- Some toxins are turned on by genetic regulation.
- Toxin tests are also available for nodularins, commonly produced by marine/brackish cyanobacterium called *Nodularia* (uncommon to New England).
- BMAA, DAB toxins (neurotoxins) have been associated with nearly all cyanobacteria.
- Dermal-toxins, causing rashes on skin can occur with most cyanobacteria.

Table created by Amanda Murby McQuaid

Table 2: Cyanotoxins and common modes of action (modified from *Handbook of Cyanobacteria Monitoring and Cyanotoxin Analysis, First Ed. 2017*).

Cyanotoxin	Mode of action and/or symptoms
Microcystins (nearly 100 variants)	Hepatotoxic, targets the liver and digestive organs, tumor promoting, inhibition of protein phosphatases. Acute gastroenteritis, chronic tumor promotion.
Nodularins (similar in structure to microcystins)	Similar to microcystins, but not as toxic and common in brackish or marine systems.
Anatoxin-a	Neurotoxic, inhibits acetylcholine receptors (neurotransmitter). Fast-acting and may cause seizures or death (i.e. common for dogs or other animals to ingest and die).
Anatoxin-a (S)	Neurotoxic, similar to anatoxin-a
Saxitoxins	Neurotoxic, blocking voltage gate of sodium ion channels. More common to marine organisms.
Cylindrospermopsins	Toxic to multiple organs, neurotoxic and genotoxic, affecting neurons and genes.
Lyngbyatoxins	Tumor promotion
BMAA/DAB	Neurotoxic, chronic exposure may be linked to neurodegenerative diseases such as ALS. (Though individuals may have a genetic precursor).

Note:

- Dermal-toxins, causing rashes on skin and can occur with most cyanobacteria. Usually depends on the individual in contact.
- Synergistic effects of the cyanotoxins may also occur.
- Many of the cyanotoxins cause gastroenteritis-like symptoms, while others may cause seizure-like or possibly neurodegenerative symptoms.
- Exposure can occur through drinking, food, dietary supplements, inhalation, and/or by dermal contact, and has occurred by haemodialysis (with contaminated water).

Table created by Amanda Murby McQuaid

EPA Methods for Cyanobacteria Toxin Analysis

EPA Method 544 - LC-MS/MS – JCLC does not perform this analysis. See Table 3.

Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry

EPA Method 545 - LC/ESI-MS/MS – JCLC does not perform this analysis. See Table 3.

Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry

EPA Method 546: ADDA-ELISA

Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA-Enzyme-Linked Immunosorbent Assay

Table 3: Labs Conducting LC/MS and ADDA-ELISA Toxin Analysis using EPA Methods 544/545/546

The Rhode Island State Health Labs and Greenwater Labs have indicated they can conduct toxin testing in a two- to three-day turnaround time for properly submitted samples. Other labs listed by US EPA are available for similar services. Consult US EPA's list of Laboratories that Analyze for Cyanobacteria and Cyanotoxins.

RHODE ISLAND STATE HEALTH LABORATORIES	Evan K. Philo Principal Lab Scientist/Food Testing Coordinator 401-222-5553 Evan.Philo@health.ri.gov	RI State Health Laboratories RI Dept. of Health 50 Orms Street Providence, RI 02904	Combined EPA 544/545 methods: $250/sample EPA Method 546: $50	**Sample Protocol:** Amber glass bottles Keep cold. **Shipping Protocol** Ship on ice
GREENWATER LABORATORIES	(386) 328-0882 Greenwaterlab.com	205 Zeagler Dr. Palatka, FL 32177	EPA 545 method: $420/sample EPA 546 method: $240/sample Shipping Cost: $100 Lab DOES NOT do EPA Method 544	**Sample Protocol:** Amber glass bottles Keep cold **Shipping Protocol** Ship on Ice

Cyanobacteria and Cyanotoxin Sampling for Public Water Systems

This sampling protocol outlines how to collect cyanobacteria and cyanotoxin samples at public water systems source waters, finished waters, and other sampling locations. This document should be used when responding to suspected blooms of harmful cyanobacteria in conjunction with *NH Department of Environmental Services CyanoHAB Response General Protocol for Public Water Supplies*. This protocol does

not address sample collection for site specific monitoring plans. Consult with NHDES-JCLC, Harmful Algal and Cyanobacterial Bloom Program, on recommendations for a routine cyanobacteria monitoring plan, or refer to EPA's Cyanobacteria Monitoring Collaborative at cyanos.org.

Safety Precautions

Safety must come first when sampling for cyanotoxins. Be cautious and wear protective gear such as gloves if handling a suspected harmful cyanobacteria bloom. Precautions should be taken to avoid mouth and eye contact. Consider wearing eye protection and a mask to further prevent exposure. Chest waders should also be worn if collecting a cyanotoxin sample when wading off the shore to protect skin from contact with cyanotoxins. Always wash your hands and rinse thoroughly after handling.

Recommended Supplies

For cyanobacteria and cyanotoxin sampling at public water systems, the recommended supplies include:

General Supplies

- Disposable gloves or re-usable arm-length gloves
- Goggles and a mask for collection of blooms that are exceptionally concentrated and as a general precaution
- Cooler with packed ice for sample storage under 12 hours and/or refrigerate if up to 24 hours until delivery. (Do not allow bottles to float in warm water or melted ice water).

Analytical Method Specific Supplies

For cyanobacteria screening and toxin sampling for Microcystins/Nodularins at NHDES JCLC:

- HDPE (high density polyethylene), brown/amber bottles of at least 125 mL capacity.
- Bottles will be supplied upon request by NHDES JCLC – (603) 848-8094 - Cyanobacterial Bloom Hotline

For cyanobacteria toxin sampling for lab analysis to be done using EPA Methods 544/545 at Rhode Island Public Health Labs or Greenwater Labs:

- 500-mL amber glass bottles (1-2) fitted with polytetrafluoroethylene (PTFE)-lined screw caps and EPA Method specific preservatives
- Bottles and preservatives will be supplied upon request by NHDES DWGB - contact:
    - Liz Pelonzi - Source Protection Specialist – 603-271-3906; ann.pelonzi@des.nh.gov; or
    - Pierce Rigrod – Supervisor, Source Water Protection Program – 603-271-0688; Pierce.Rigrod@des.nh.gov

Sample Collection, Preservation, Shipment and Storage

Sample Collection Procedure for EPA Methods 544 and 545

- Open the cold water tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 min). Collect samples from the flowing system. Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.
- After collecting the sample, cap the bottle and agitate by hand until preservative is dissolved. Note that 2-chloroacetamide is slow to dissolve especially in cold water. Samples must be chilled during shipment, but should not be frozen.

Sample Collection Procedure for EPA Method 546

- In the field, open the tap and allow the system to flush for approximately 5 minutes. Fill each bottle, taking care not to flush out the sodium thiosulfate, and invert several times to mix the sample with the reducing agent. Sample must be chilled during shipment but should not be frozen.

Label and store water samples properly

- Label bottle(s) to include:
  - Waterbody and location
    - Intake,
    - Finished water,
    - Or other location within source
  - Date and time
  - Type of sample
- Place in packed ice in cooler (bottles should be kept at or below 10°C) **DO NOT FREEZE for cell count and Identification (unless finished water).**

Sampling Methods

- **Discrete samples** - samples that are collected from a discrete, specific location or depth within the water column. A collection device, such as a Van Dorn Water Bottle, can be deployed within the water column to a specific depth. Recommended for:
  - Open water (at a specific depth)

- **Grab samples** - samples that are collected from a sample tap or by submerging a bottle in water at an attainable location by hand. Sample by submerging bottle slowly through the water in a u-shaped orientation. Recommended for:
  - Water surface
  - Beach or shoreline at knee depth (or about 1 meter)
  - Raw or finished water (from a sample tap)

- **Integrated tube samples** - samples that are collected using an integrated tube sampler which represents a column of water from 0-1 meter at the shore or 0-3 meters from deeper water. Collection depths can vary. These samples are integrated and well mixed, typically most representative of the surface water as a whole. Recommended for:
    - Samples collected for the *EPA Cyanobacteria Monitoring Collaborative*

- **Surface skim** - using a collection bottle to skim the surface of the water tension. Recommended for:
    - Dense surface bloom or shoreline accumulation

Sampling Procedure for submittal to New Hampshire Department of Environmental Services (NHDES) Jody Connor Limnology Center (JCLC) for analysis:

1. Take a photo of the bloom if possible and submit to Harmful Algal and Cyanobacterial Bloom Program Coordinator at JCLC (HAB@des.nh.gov) or use the Bloom Watch App (cyanos.org)
2. Collect a sample(s) from the options listed below, using a 125mL brown HDPE bottle(s), or by using methods suggested by EPA Cyanobacteria Monitoring Collaborative (CMC) Quality Assurance Project Plan (QAPP). *NHDES JCLC will advise water systems on which method is recommended.*
    a. Discrete water sample
    b. Grab sample
    c. Integrated tube sample
    d. Surface skim
    **Avoid collecting samples from areas where the bottom sediment has been disturbed.*
3. Label and store water samples properly
    a. Label 125 mL brown HDPE bottle(s) to include:
        i. Waterbody and location (coordinates if possible)
            1. Bloom
            2. Open water away from bloom)
            3. Intake, Raw water
            4. Finished water
            5. Or other location within source
        ii. Date and time
        iii. Type of sample (indicate if this was a surface bloom or other)
        iv. How sample was stored (eg. on ice, frozen, no preservative)
    b. Place in packed ice in cooler (if delivering within 12 hours of event)
    Or
    c. Freeze sample (if storage on ice or refrigeration will be longer than 24 hours) until delivery to NHDES-JCLC. *Avoid filling to the top to allow for freezing expansion- fill about 2/3rd of bottle or to the neck.*

4. Bring samples to NHDES - JCLC, 29 Hazen Dr., Concord, NH during business hours (8 am-4 pm) or by prior arrangement (see contact information in *CyanoHAB Response General Protocol for Public Water Supplies*.)
    a. At JCLC samples may be analyzed for:
        i. Cyanobacteria ID and enumeration
        ii. Microcystins/nodularins by ELISA methods (1-2-day turnaround)
5. Fill out a requisition form at the JCLC for confirmation of sample delivery and details of bloom event with appropriate contacts.
    a. Your name, contact and concern
    b. Location – waterbody, beach or specific bloom area or depth
    c. Date, time and weather
    d. Details or description of bloom (photo submission if possible) – surface scum or throughout lake, surface area or magnitude of area, color or odors, etc.
    e. How it was collected and stored prior to delivery
    f. Station ID (ID will be provided by JCLC upon confirmation of the location sample was taken)

Sample analysis for cyanobacteria ID and enumeration, and Microcystin-ADDA ELISA quanitplate by NHDES-JCLC will be free of charge for 2020.

If additional toxin analysis for Microcystins, Nodularin, Cylindrospermopsin and Anatoxin-a using EPA Methods 544 and/or 545 is required, the NHDES-DWGB will request the system take additional samples. Laboratories that conduct these methods are listed in Table 3 above.

For samples submitted to alternative labs for EPA 544/545/546 methods, please follow the instructions provided in the above sampling and shipping procedures beginning on page 8.

How to Make an Integrated Tube Sampler

MATERIALS:

Tygon tubing (0.5-inch diameter; available in most hardware stores)
Plastic bottle (0.5 liter wide-mouth Nalgene works well)
Eyebolt, nut and washers
Ballast material (ready-mix cement)
Silicone adhesive
Small hose clamps

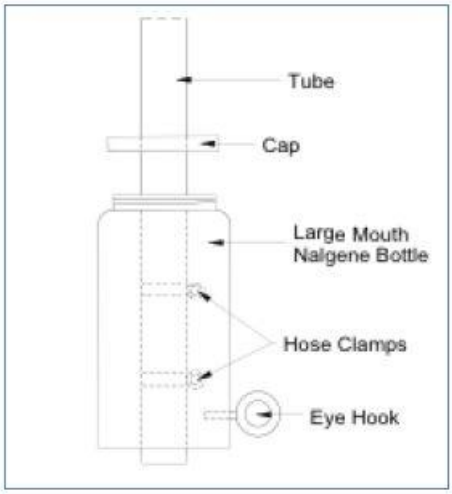

Figure 1 Basic design of weighted end of integrated tube sampler; tube length and diameter can vary but needs to be noted when samples are collected.

CONSTRUCTION:

1. Cut the Tygon tubing to desired length (the length of the tubing should exceed the sampling depth by at least 0.5 meters). Mark the tube at 0.5 meter intervals using a waterproof permanent marking pen.
2. Drill a hole the size of the tube in the cap and bottom of a Nalgene bottle.
3. Place cap on tube, followed by the hose clamps positioned about 4 to 6 inches from end of tube. Tighten the clamps so that they will not slip on tube.
4. Place the bottle so that approximately 2-3 inches of tube protrudes through the bottom of the bottle. Slide the cap up the tube such that the bottle is open.
5. Screw and secure that eyehook into bottle, using a nut and washers.
6. Prepare cement mix according to instructions and pour in the bottle around the tube until bottle is filled to the bottom of the neck.
7. Slide cap in place and screw on tight. Apply silicone to seal around the tubing.
8. Allow tube sampler to dry for 24 hours before use.
9. Attach a calibrated line to the eyebolt for retrieval of the sampler.

OPERATION:

1. Lower tube to desired depth using markings on the tube to judge the depth
2. Crimp the tube above the water.
3. Retrieve the tube using the line attached to the bottle to prevent loss of water from the tube
4. Place the open end of tube (protruding from the bottle) into the sample jar.
5. Note that if samples from more than one location are being combined (e.g. for cyanobacteria sampling), be sure to select a container of adequate volume.
6. After all sites are combined, *mix the container thoroughly* and pour into HDPE plastic sample container (0.5-1 liter), allowing room for expansion if samples will be frozen.
7. Place sample in cooler with ice until frozen or processed for microcystin analysis.