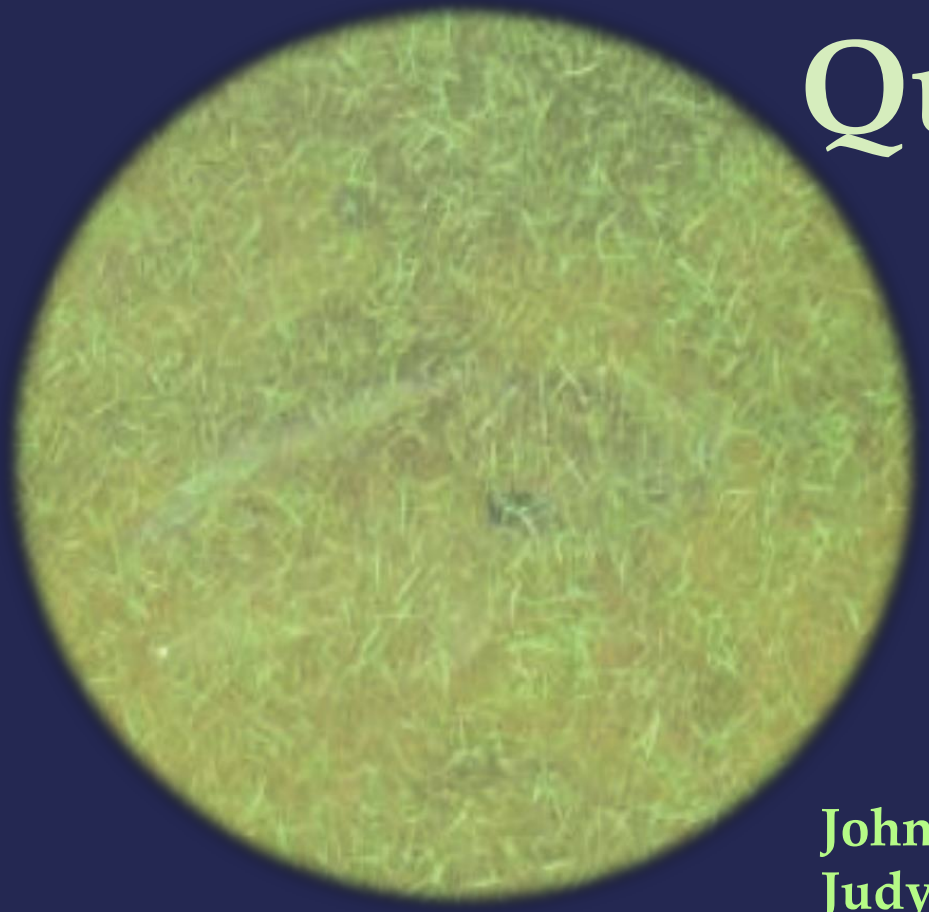
Detection Techniques used for Microcystin Quantification



Water Analyst Workshop
May 14, 2015

Johnna Birbeck, Ph.D., Wayne State University
Judy Westrick, Ph.D., Wayne State University

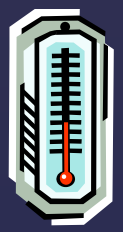
Cyanotoxin Overview

- Cyanotoxins are produced by cyanobacteria
  - Blue-green algae
  - Prevalent throughout the world
- No way to distinguish a toxic bloom from a non-toxic bloom
- Cyanobacteria release toxin upon cell death or lysis
  - May persist in the water source for weeks to months
- Most common toxin formed from cyanobacteria are the microcystins
- No relationship between taste and odor events in drinking water and cyanotoxin occurrence

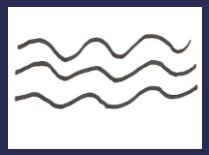
USEPA: (http://iaspub.epa.gov/tdb/pages/contaminant/contaminantOverview.do?contaminantId=-1336577584)
Image courtesy Tom Archer / Michigan Sea Grant. (http://www.circleofblue.org/waternews/2014/world/choke-point-index-great-lakes-drinking-water-fouled-by-toxic-algae/)

Algal blooms are caused by

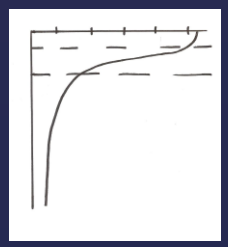
Nutrient Load



Water Temperature



Flow



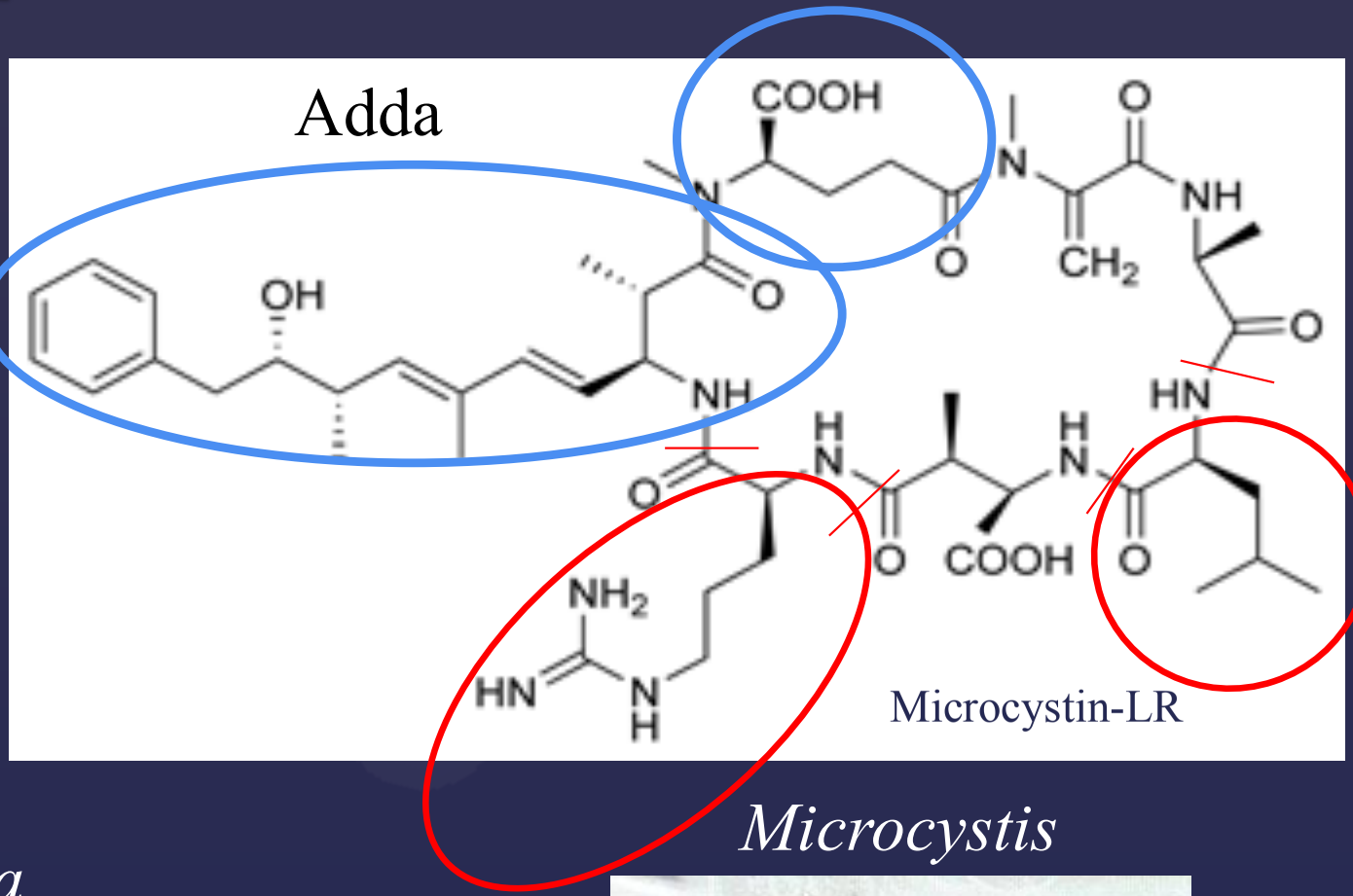
Thermal Stratification

Rainfall

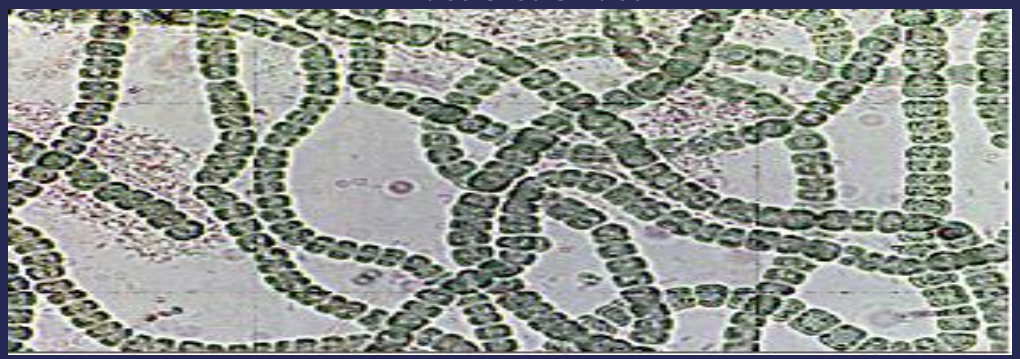
Routes of Exposure

Microcystin

- Microcystin LR LD_{50} 50 µg/kg
- Inhibits serine/threonine protein phosphatase 1 and 2A
- Hepatotoxic



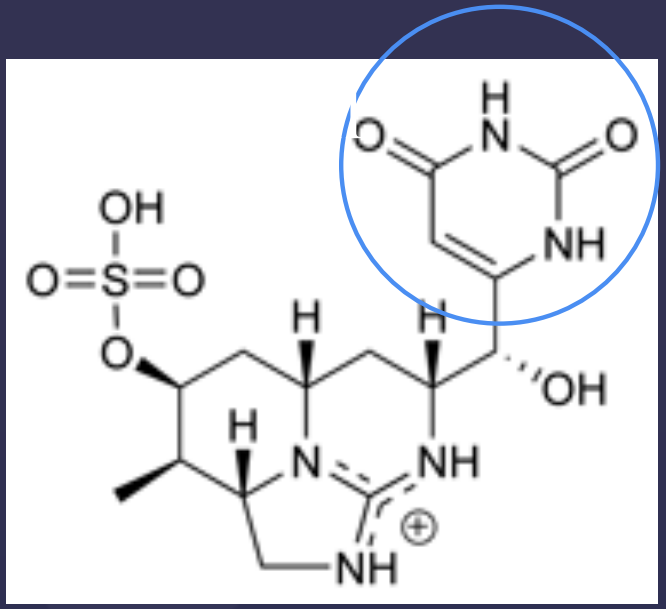
Anabaena



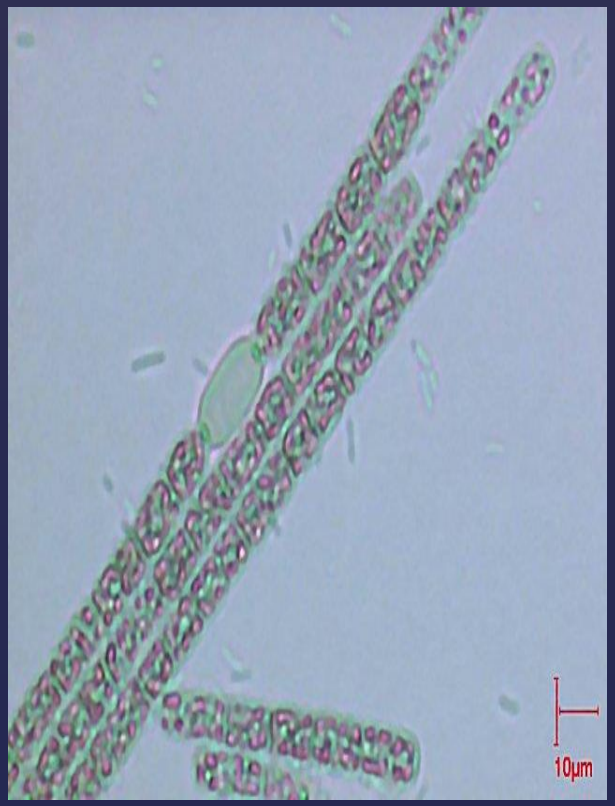
Microcystis

Cylindrospermopsin

- LD_{50} 50 µg/kg
- Hepatotoxic
- Cytotoxin

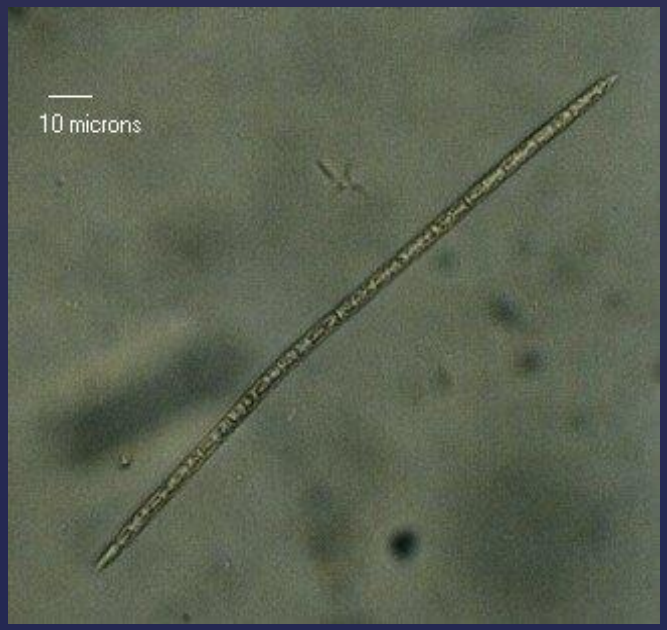


Aphanizomenon

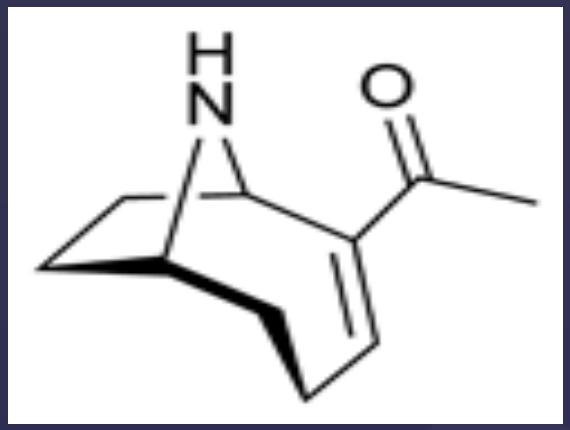


Cylindrospermopsis

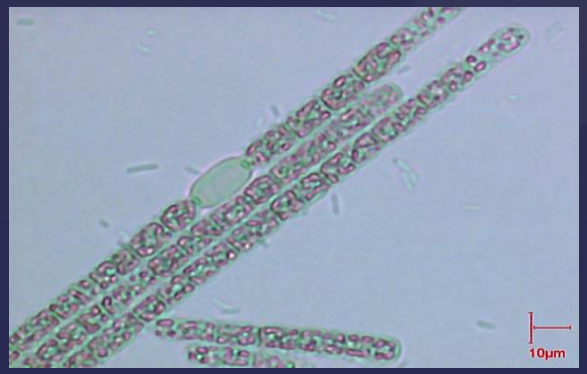
Cylindrospermopsis



Anatoxin-a



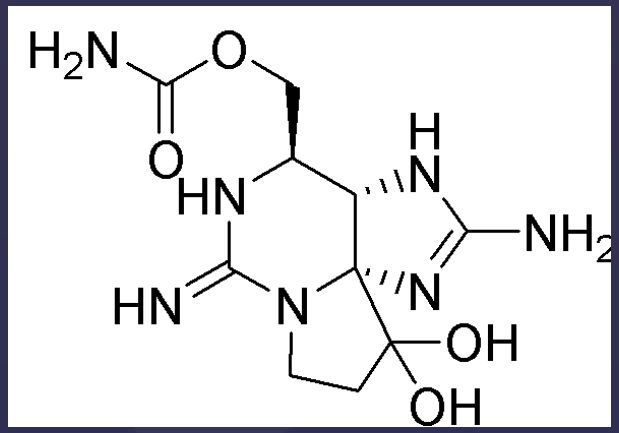
- LD_{50} 50 µg/kg
- Neurotoxin
- Inhibits acetylcholine receptor



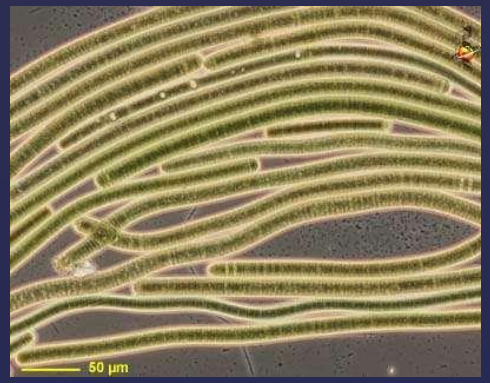
Aphanizomenon

Anabaena sp.

Saxitoxin

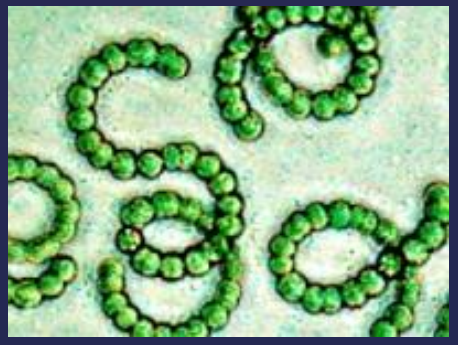


LD_{50} 9 µg/kg

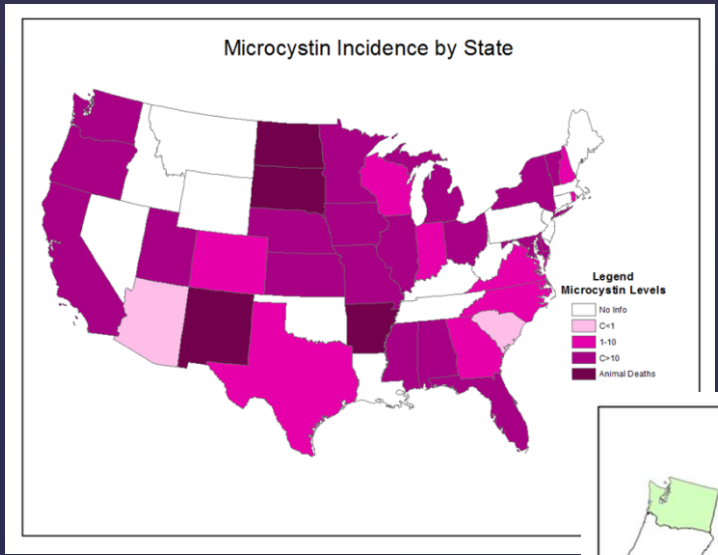


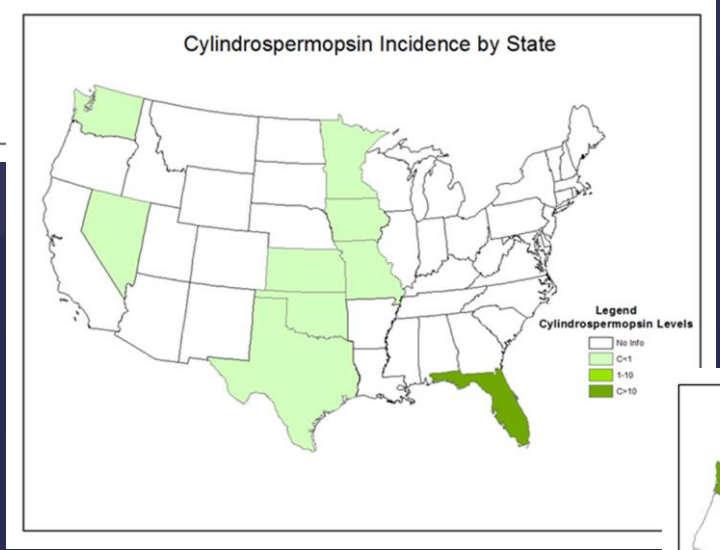
Lyngbya wollei

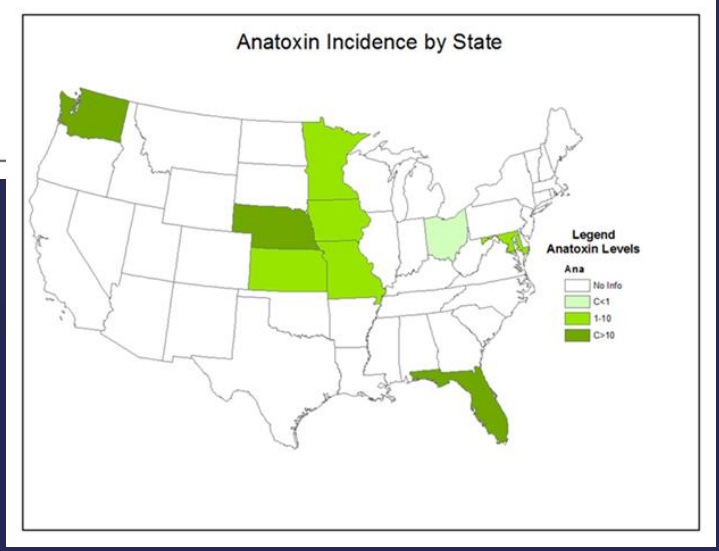
Anabaena circiralis



Cyanotoxin Occurrence







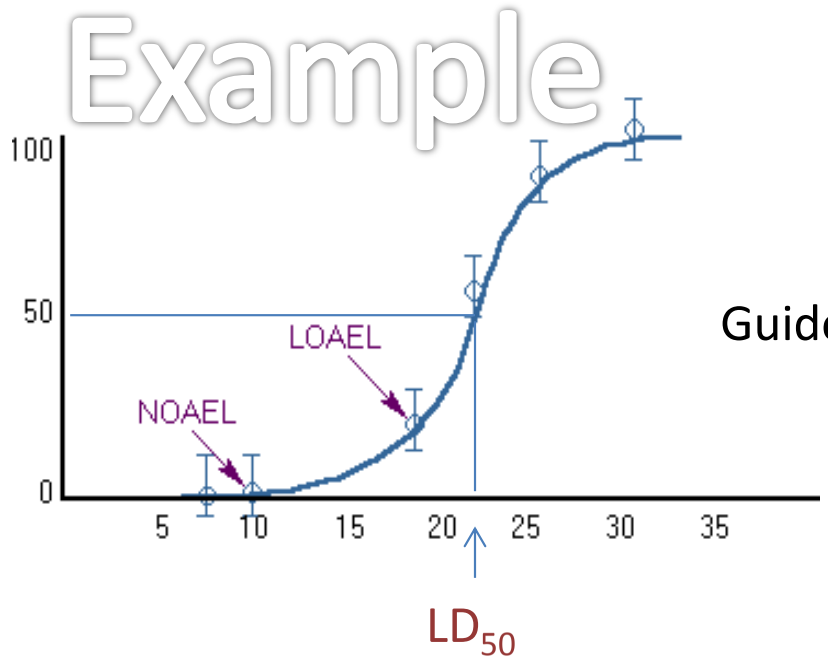
Are cyanotoxins regulated in drinking water?

- No federal regulations for cyanobacteria/cyanotoxins in drinking water
- Safe Drinking Water Act (SDWA) requires EPA to publish list of unregulated contaminants present or expected to be detected in public water systems
  - Contaminant Candidate List (CCL)
  - EPA uses CCL to prioritize research to determine if contaminant has sufficient data to meet regulatory determination criteria specified in SDWA
  - As of 2012, three cyanotoxins are listed on the CCL 3:
    - anatoxin-a
    - microcystin-LR
    - cylindrospermopsin

US EPA Health Advisory

- Microcystin
  - 10-day Health Advisory recommended concentrations for total microcystins are:
    - 0.3 µg/L for children younger than school age
    - 1.6 µg/L for all other age groups
- Cylindrospermopsin
  - 10-day Health Advisory recommended concentrations for cylindrospermopsin are:
    - 0.7 µg/L for children younger than school age
    - 3.0 µg/L for all other age groups

How Drinking Water Health Advisory Levels are Determined



$$RfD = \frac{(NOAEL)}{\text{safety factors}}$$

$$\text{Guideline Value} = \frac{(TDI \text{ or } RfD)(\text{body weight})(\text{portion})}{L}$$

- Tolerant Daily Intake (TDI, WHO) and Reference Dose (RfD, USEPA) use the No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL).
- Safety Factors = interspecies, intraspecies, and others (up to 10,000 but many standards are between 100 - 1000)
- Regional Difference = average body weight, how much people drink

For detailed information, see Ch. 4 of the WHO document *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*.

Analytical Techniques used for the Detection of Cyanotoxins

- Enzyme Linked Immunosorbant Assay (ELISA)
- High-Performance Liquid Chromatography with Photodiode Array Detection (HPLC-PDA)
- LC-Mass Spectrometry Detection (LC-MS/MS)
- Nuclear Magnetic Resonance (NMR)
- Thin Layer Chromatography (TLC)
- Protein Phosphatase Inhibition Assay (PPI Assay)

Analytical Techniques used for the Detection of Cyanotoxins

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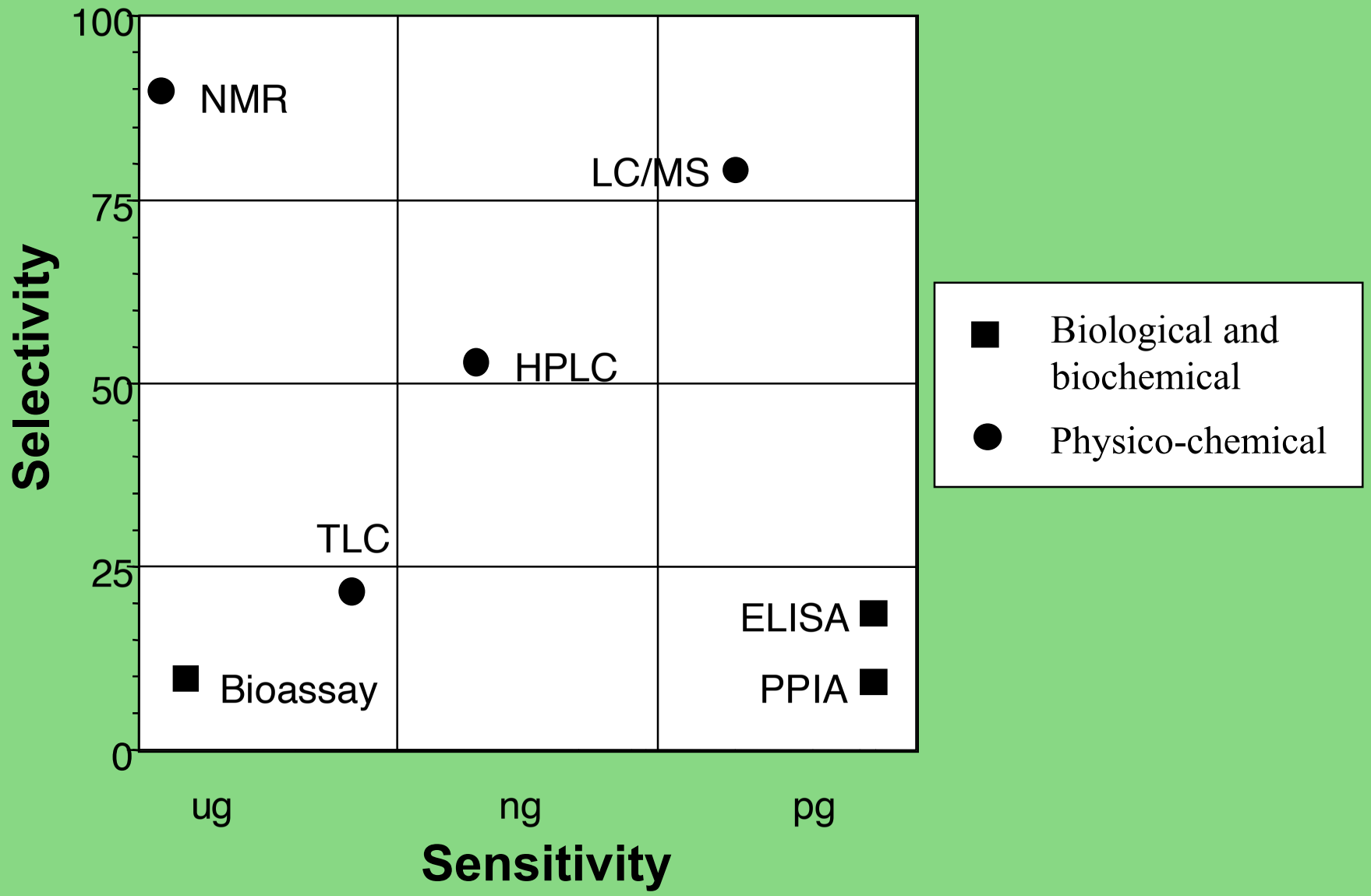
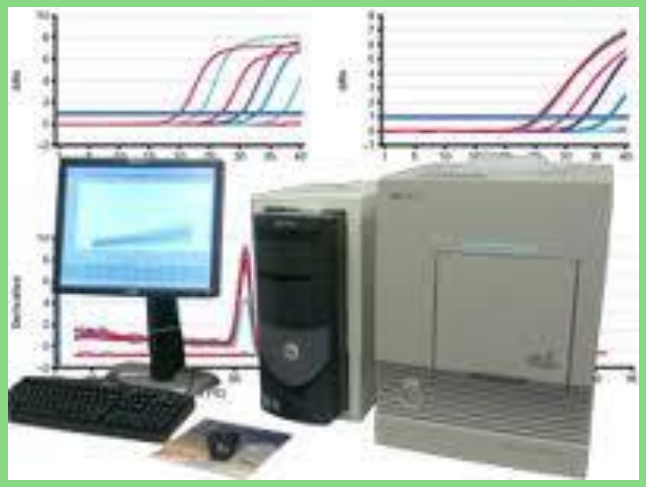
Selectivity and Sensitivity Relationships between Analytical Methods for Microcystins



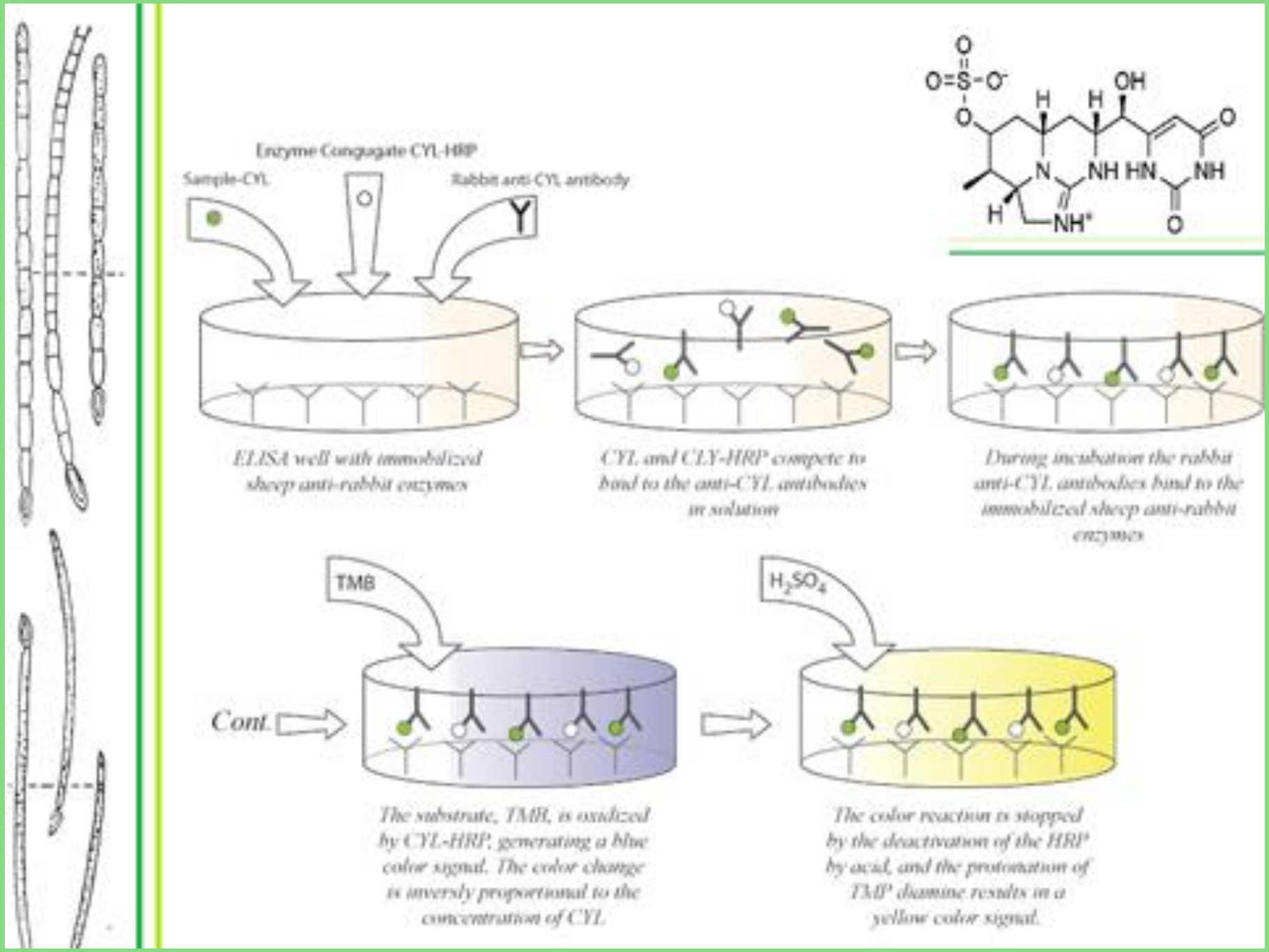
Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. (1999)

Screening Tools

- ELISA
  - Antibody, antigen
- Receptor Binding
  - Based on a receptor
- Enzyme Inhibition
  - Inhibiting an enzyme from its function
- qPCR
  - Is the gene present?



ELISA – How does it work?



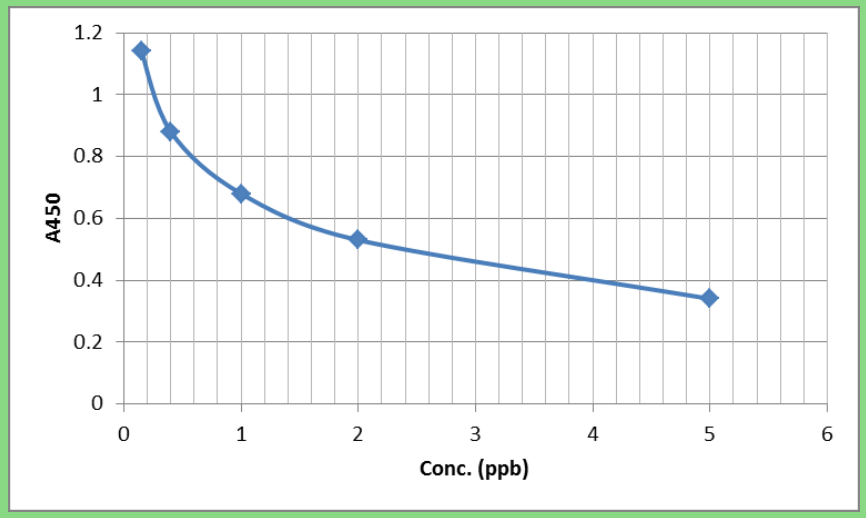
Types of ELISA kits and their detection ranges

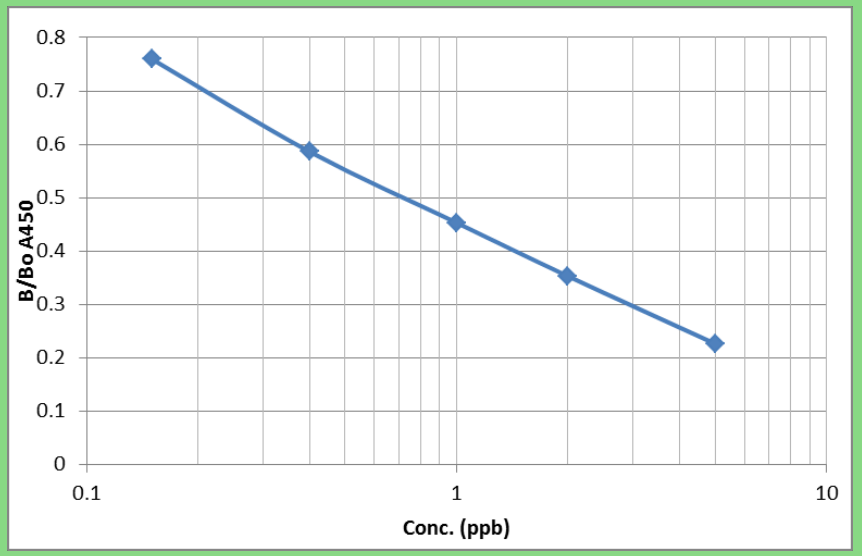
- Microcystin (and Nodularin)
  - Competitive ELISA 96-well microplate
    - 0.16 – 2.5 ppb (EnviroLogix)
    - 0.15 – 5 ppb (Abraxis)
  - Competitive ELISA test tube (EnviroLogix)
    - 0.5 – 3 ppb
- Cylindrospermopsin
  - Competitive ELISA 96-well microplate
    - 0.1 – 2 ppb (Beacon)
    - 0.05 – 2 ppb (Abraxis)
- Saxitoxin
  - ELISA 96-well microplate (Abraxis)
    - 0.015 – 0.2 ppb

***All ELISA protocols listed here state that a positive result should be further tested *** with a quantitative analytical procedure such as HPLC, LC/MS, etc.

ELISA – A Screening Tool

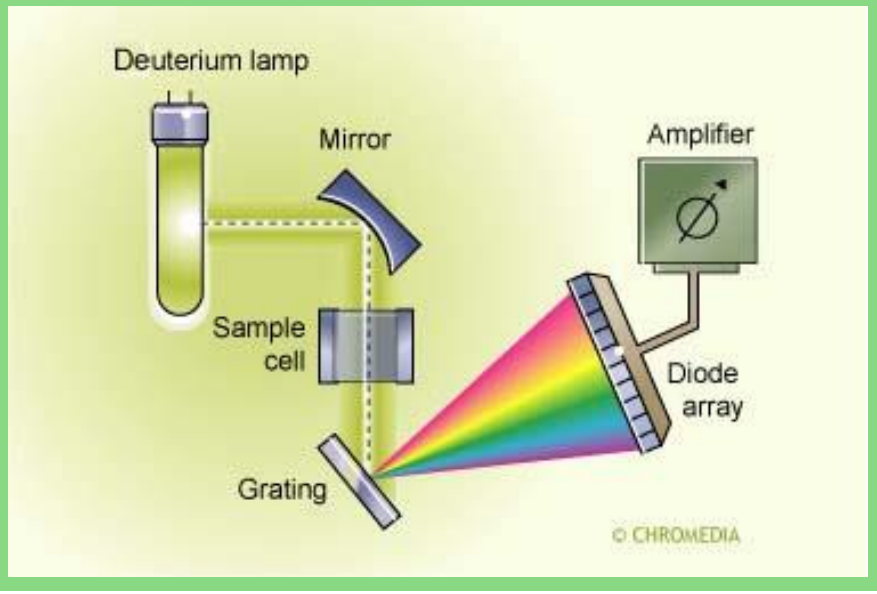
- Why are ELISA assays only a screening tool?
  - Nonlinear standard curve
  - Cross reactivity
  - Not measuring the cyanotoxin directly
    - Total microcystins



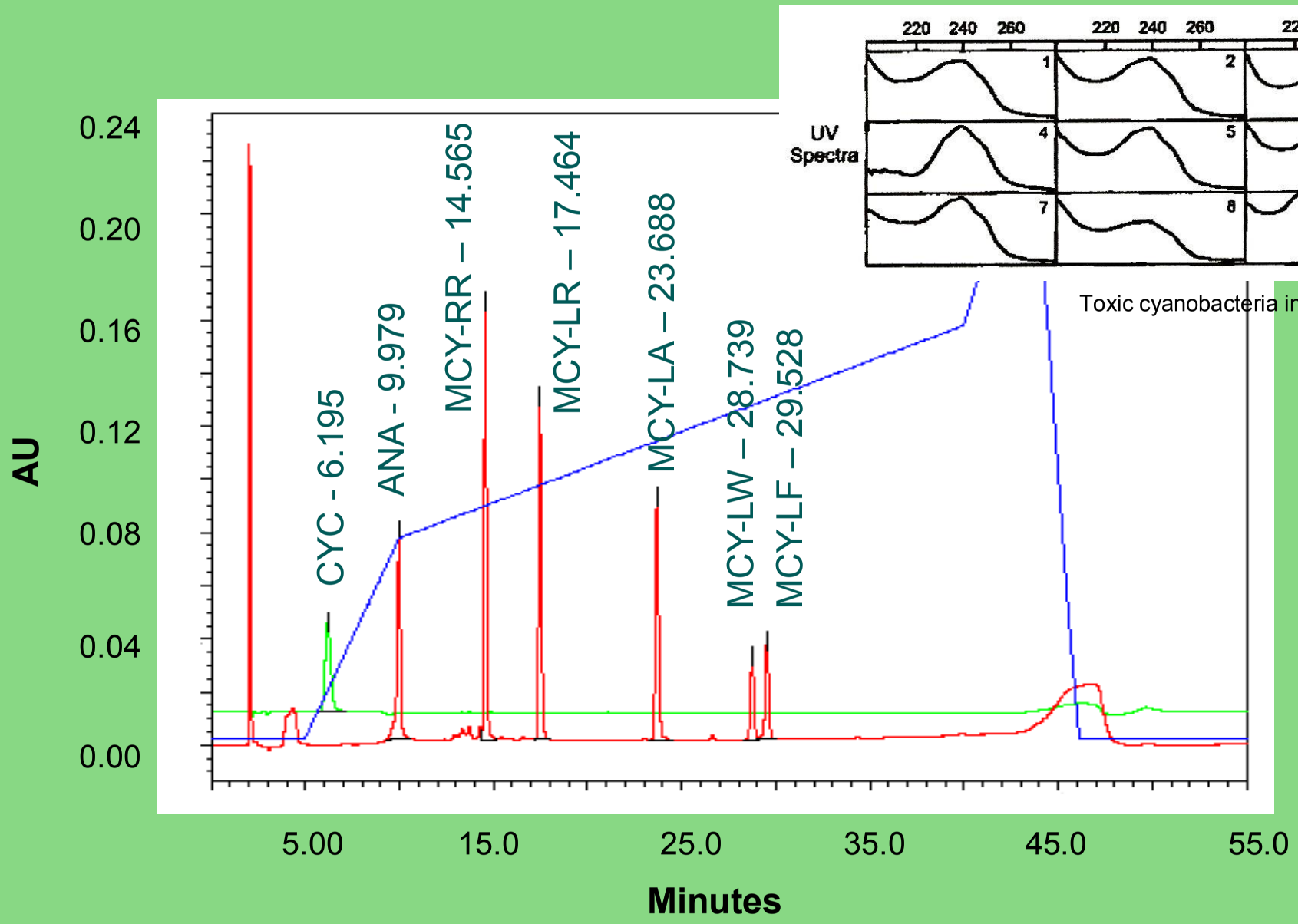


HPLC-PDA detection

- Reversed-phase C18 columns
- Detection of microcystins at 238 nm
- Gradient analysis using acetonitrile and water both with the addition of trifluoroacetic acid
  - TFA used to protonate carboxylic acids and acts as an ion paring reagent to limit basic interactions with the column
- Typical run times ~50-60 minutes



Separation of the Cyanotoxins by HPLC-PDA



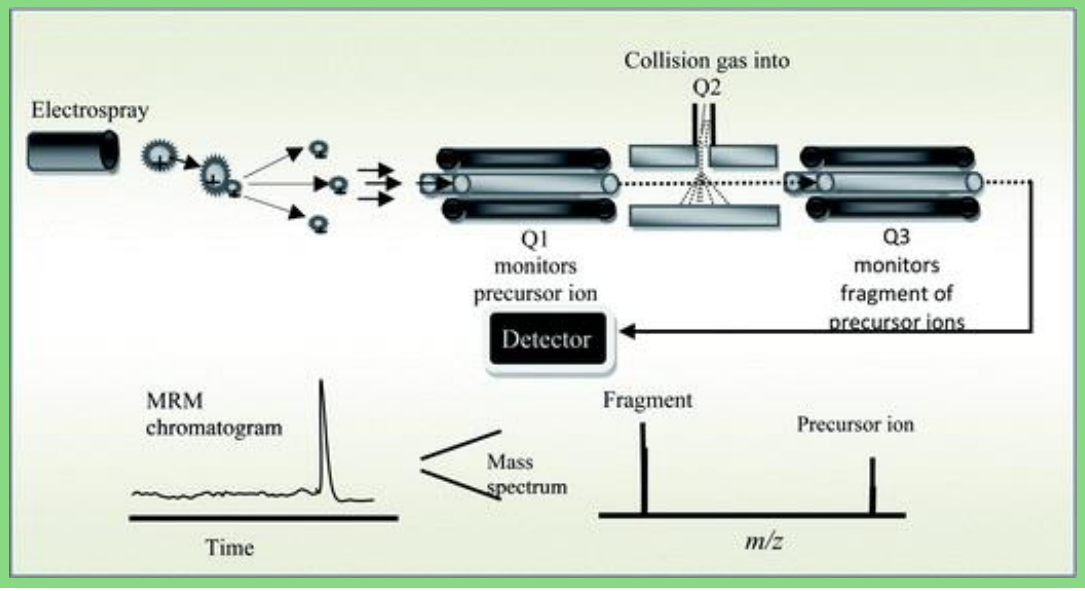
Toxic cyanobacteria in water 1999

HPLC-PDA

- Pros
  - Able to identify multiple different cyanotoxins in one sample run
  - Linear standard curves
- Cons
  - Long sample run times
  - Concentration range using PDA detection is between 0.05 – 2 ppm
    - Samples must be concentrated

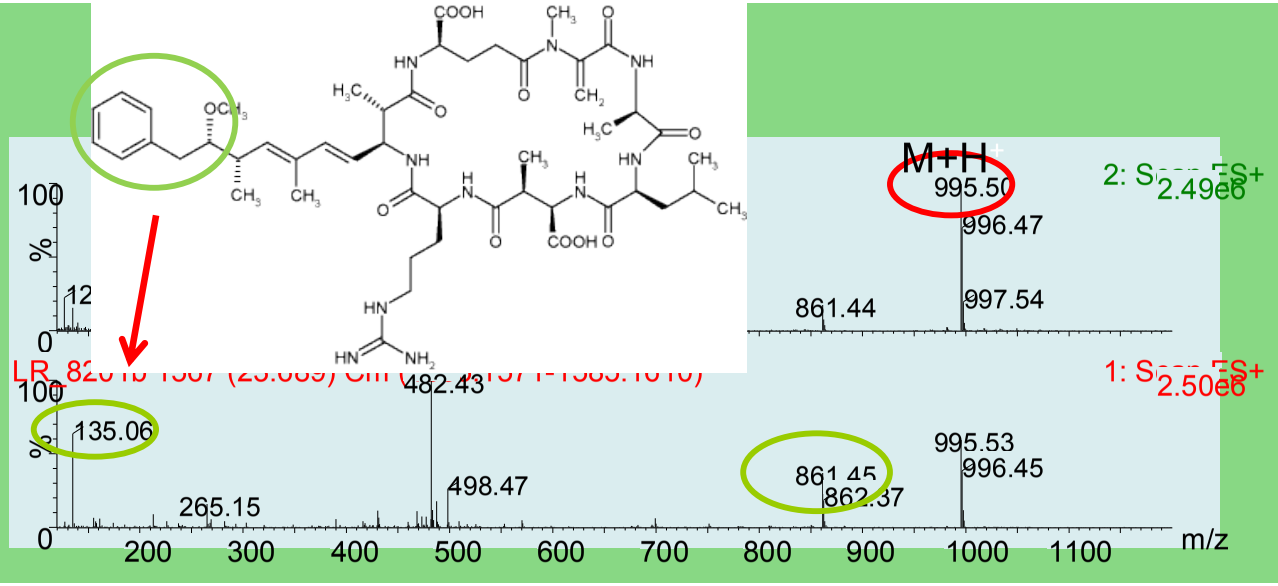
LC Mass Spectrometry

- UPLC and HPLC gradient methods
- Formic acid used instead of TFA for protonation
- Can be used in tandem with PDA detectors
- Multiple different types of MS can be utilized
  - Q-Tof for high resolution
  - MS/MS for further separation of ions



Dahab and Smith (2012)

UPLC-MS/MS Chromatograph



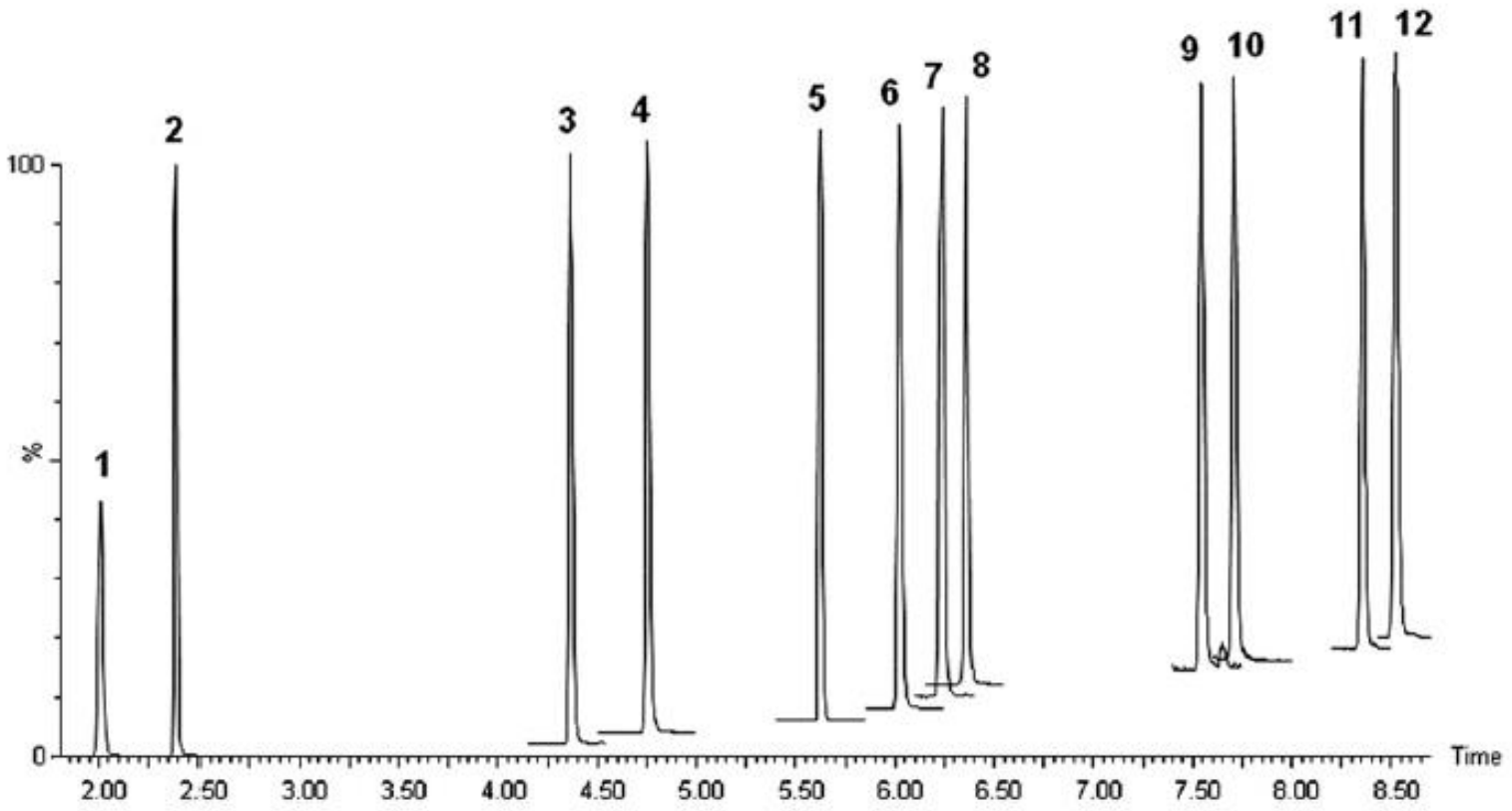


Fig. 1. UPLC/MS/MS separation of Components of interest. Peaks: 1 = Cylindrospermopsin, 2 = Anatoxin-a, 3 = Cyclo (Arg-Ala-Asp-D-Phe-Val) (IStd), 4 = [Leu^5]-Enkephalin (IStd), 5 = Microcystin RR, 6 = Nodularin, 7 = Microcystin YR, 8 = Microcystin LR, 9 = Microcystin LA, 10 = Microcystin LY, 11 = Microcystin LW, and 12 = Microcystin LF.

Oehrle et al., (2010)

UPLC-MS/MS

- Advantages
  - Very selective
  - Reaches ppt detection limits
  - With UPLC, shorter run times can be achieved
  - Identify unknown microcystins in samples
  - Small amounts of sample used
- Disadvantages
  - Expensive
    - Instrumentation, solvents, and consumables
  - Extensive training
  - Destructive technique

Looking for a Lab -FAQs

- Sampling – Talk about this
- Request a SOP or notes on the method – don't assume
- What screening tool or analytical instrument is the laboratory using – don't assume
- What are the MRL or MDL? – don't assume
- How long will it take to get your results?
- Ask about their QA/QC
- Will the QA/QC be summarized on the report?
- Always keep a duplicate sample in the lab

How to Manage a Cyanotoxin Event

Be technically prepared

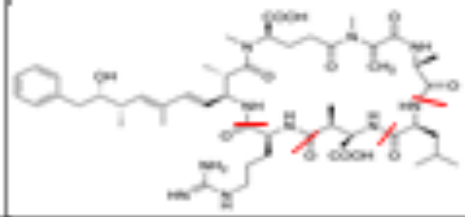
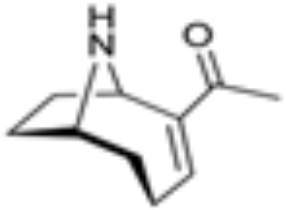
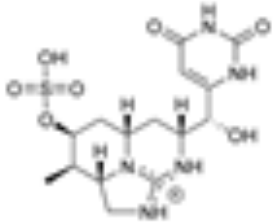
- Catch the bloom as early as possible
- Know how to manage source and adjust treatment
- Know how to quantify toxins

Be ready to communicate

- With the public
- With the press


Thank You - Questions?
A man of wisdom delights in water.
- Confucius

Summary of Cyanotoxins

Toxin	Structure	Organ	Genera
Microcystin		Liver (possible carcinogen)	*Microcystis* *Anabaena* *Planktothrix* *Anabaenopsis*
Anatoxin-a		Neurotoxin (nerve synapse)	*Anabaena* *Planktothrix* *Aphanizomenon* *Cylindrospermopsis*
Cylindrospermopsin		Liver (possible kidney, genotoxic and carcinogen)	*Cylindrospermopsis* *Aphanizomenon*

Cyanotoxins and Drinking Water

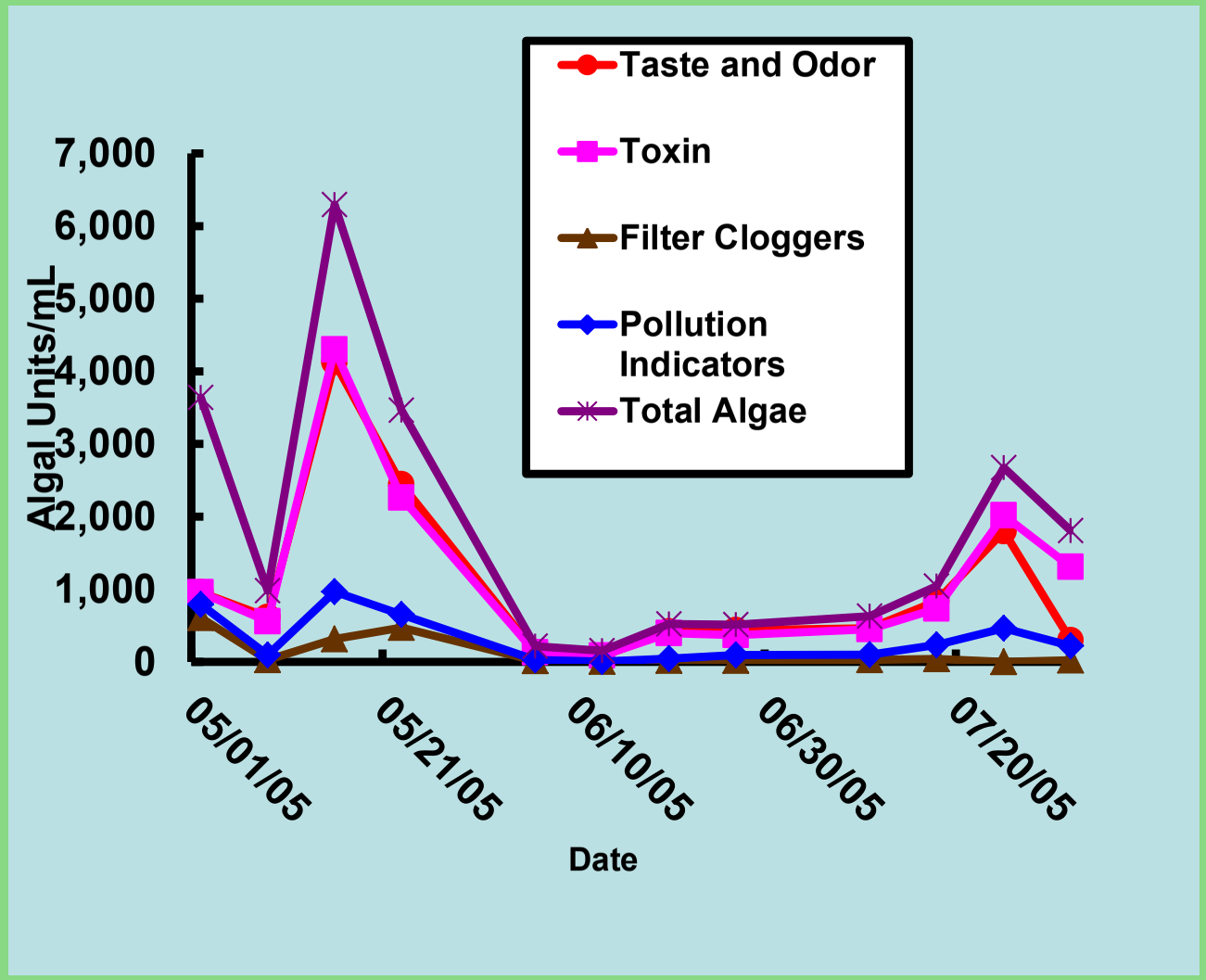
1. Understand the Problem
2. Know What Tools are Available
3. Plan How to Manage a Cyanotoxin Event

1. Understand the Problem

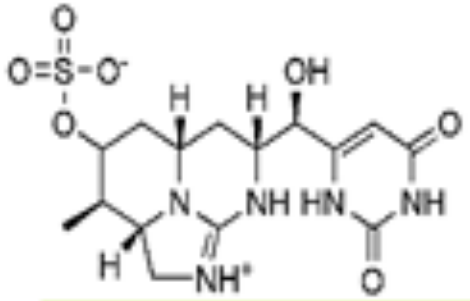
- Occurrence
- Toxicology
- Guidelines and Regulations

- USEPA – Where are we in the regulatory process?
- Cyanobacteria and their toxins
- Water Conditions
- Monitoring Tools
- Drinking Water Treatment

Early Warning Systems

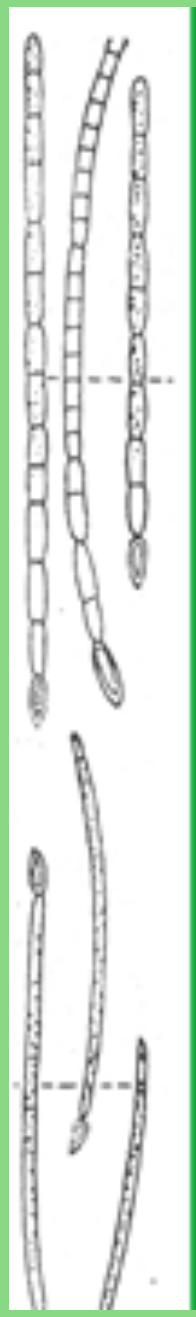


Results

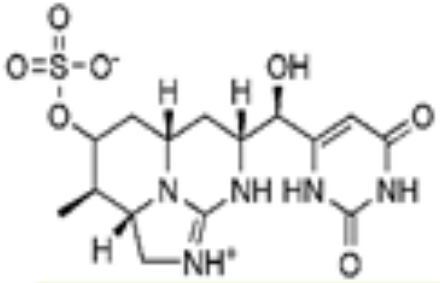


- Site Selection

Plant	Sample	Temp (C°)	pH	Turbitity	TOC mg/L	UVA @ 254 cm-1	Total Cells/mL
Florida 2	Finished	N/R	8.37	0.03	**7.2**	**0.0179**	<1
Florida 2	Raw	N/R	6.9	2.36	**15**	**0.36309**	337.5
Michigan 2	Finished	20.1	8.22	0.05	**110**	**0.35517**	<1
Michigan 2	Raw	21	7.88	0.96	**1.6**	**0.0176**	5.1
Minnesota	Finished	25	8.36	0.19	**82**	**0.08658**	<1
Minnesota	Raw	26	7.96	9.63	**4.5**	**0.07558**	2068.8



Results



- Samples
  - No false negatives or positives
  - All %CV < 15% as specified by manufacturers
  - Relative Error ranged from ±1.3-46%
  - % relative error of 87% of samples < 25%

Analytical Tools

- Sampling
- Sample Preparation
- Sceening Tools
- Analytical Instrument

Sampling

- Finished Drinking Water Samples
  - USEPA Method 544 – powder
    - Bottle - amber glass bottles fitted with polytetrafluoroethylene (PTFE)-lined screw caps
    - TRIZMA preset crystals, pH 7.0
    - L-Ascorbic Acid
    - Chloroacetamide
    - Ethylenediaminetetraacetic acid
  - Ohio EPA
    - Bottle - glass or polyethylene terephthalate glycol (PETG) container.
    - Sodium thiosulfate or Ascorbic acid
- Source, Recreational Waters, Unit Process Evaluation
  - Sample notes
    - Description of water and why the sample was taken that way. (Grab, composite, depth, location)
    - Objective – Total cyanotoxin, particulate, dissolved
    - Additives – no recommendation at this point.
    - Quantification of individual toxins vs relative concentration.
    - Sample preparation
      - Understand what is being done to your sample. Realize different laboratories have different SOPs.

Sample Preparation

- Sample Concentration
  - Filtration – only particulate microcystin
  - Lypholization – total microcystin, dissolved
  - SPE – dissolved
- Lysing Cells
  - Freeze/thaw
  - Homogenation
  - Bead Beater
  - Sonication
  - Lysing Solution