EPA/ORD Research Planning for Cyanotoxin Detection Methods in Tissues and Other Matrices

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Office of Research and Development
Center for Environmental Solutions and Emergency Response

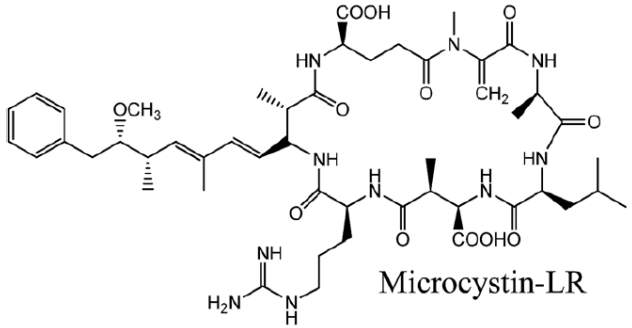
Measuring Cyanotoxins in Tissue

- Analysis methods for measuring cyanotoxins in drinking water already exist:
  - EPA Method 544 – LC-MS/MS, 7 microcystins (MCs) and Nodularin (NOD)
  - EPA Method 545 – LC-MS/MS, Anatoxin-A (ANA-a) and Cylindrospermopsin (CYN)
  - EPA Method 546 – ELISA, 'total' MCs
- Endemic HAB events in water bodies can result in significant exposure to cyanotoxins for pets, humans, and food animals. Specific toxins responsible for acute events (e.g. fish kills) may not be easily determined with existing methods
- Different toxin congeners may have changes in toxicity, bioaccumulation, and clearance, and analytical standards are not always available
- Significant challenges in extracting and analyzing toxins in tissue and other heterogeneous matrices such as soils and sediments relative to drinking water
- Goal: Develop an extraction and analysis workflow to measure and/or screen for a variety of cyanotoxins in tissue and other heterogeneous matrices

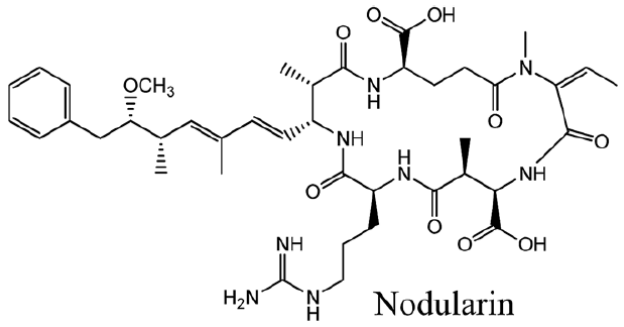
ST1

Current State of Toxin Methods for Tissue

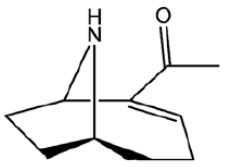
- Many single toxin/toxin class extraction/analysis methods in the literature
  - E.g. Microcystins (MCs) only, or anatoxins
  - As more toxin classes are found, running many single toxin analyses is limiting
- Relatively few studies for multiple toxin classes from tissue matrices
  - Chemical differences in toxins mean extraction efficiencies vary significantly
  - Some methods split samples through different preparations, others accept one with larger variance in recovery
- Overall conditions and techniques used vary wildly in the literature by toxin and study



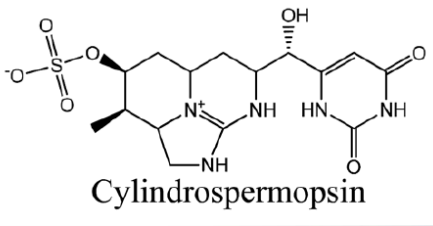
Microcystin-LR



Nodularin



Anatoxin-a

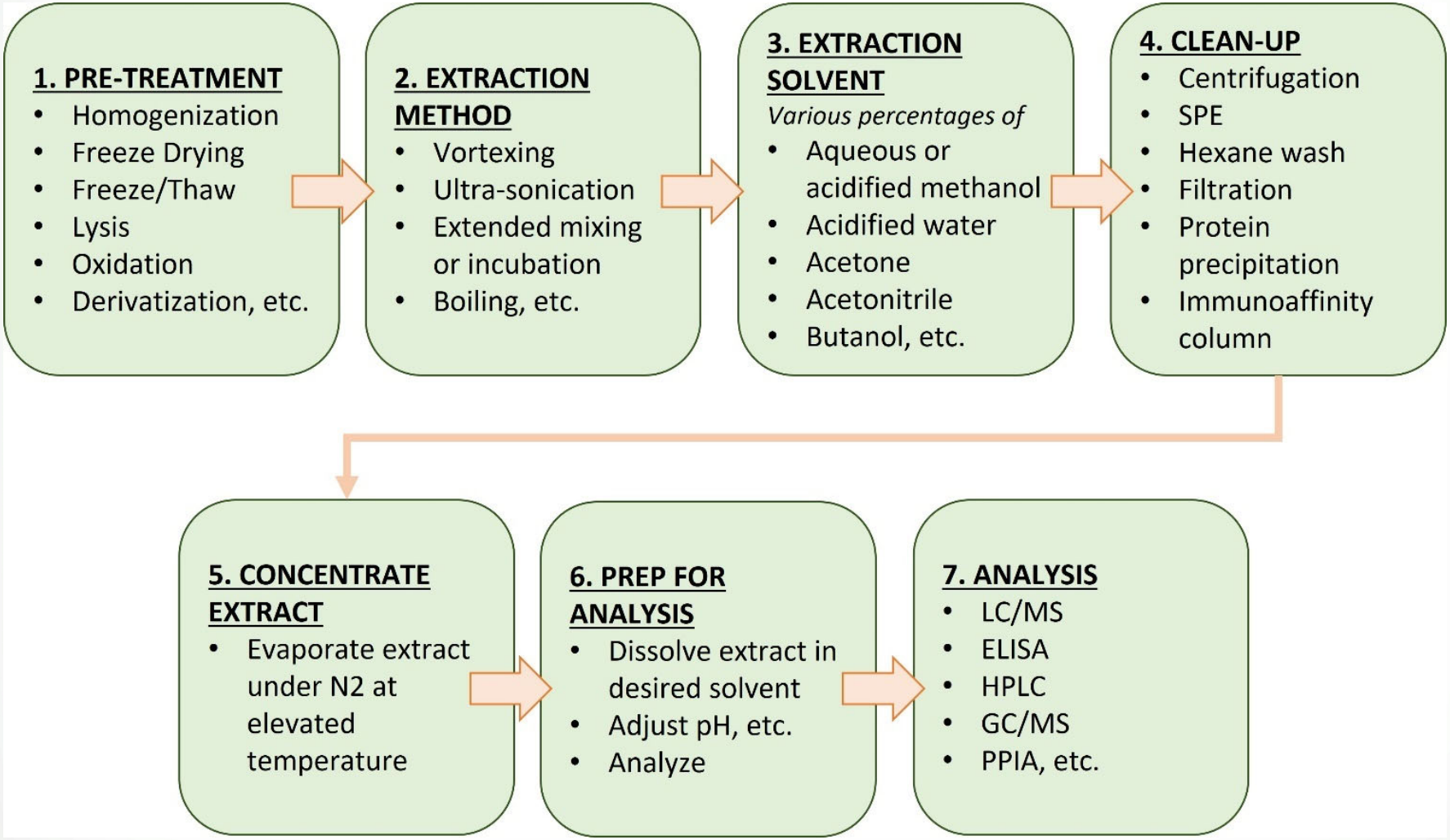


Cylindrospermopsin

Slide 3

ST1 Should I reference our Toxins review article here?
Sanan, Toby, 3/16/2022

Toxin Extraction Workflow



Proposed Extraction Optimization

- **Evaluate toxin extraction procedures from tissue, using spike/recovery studies. Each step in sample preparation/extraction can result in losses of toxin and resulting method inaccuracies**
- **Tissue preparation:**
  - Homogenization/lyophilization
  - Can lose material if samples are not sufficiently homogenous, or if toxins are in other organs
- **Extraction procedure:**
  - Can we expose the toxins to solvents for extraction without degradation or
- **Extraction solvents:**
  - Is the solvent chemistry appropriate to recover the target toxins?
  - Do we perform one extraction or sequential?
- **Cleanup:**
  - Losses through filtration/centrifugation/de-lipid steps?

Homogenization

Extraction

Cleanup

Analysis

Analytical Methods

- LC-MS/MS: Targeted screening for known toxins
  - Requires analytical standards for toxins of interest, but very accurate and sensitive
  - For some cyanotoxins this is a challenge preventing analysis, e.g. Prymnesins, less common MC congeners
- MCs, NOD, ANA-a and CYL have literature precedents for a single analysis method
  - Final tissue extracts will need to be compatible with the LC-MS/MS workflow
- LC-MS/MS: Targeted Screening With Isotope Dilution
  - Add labeled analogs of toxins to samples before analysis – Can improve method reliability, both in analysis and extraction
  - Isotopically labeled standards are only available for some cyanotoxins

EPA Research Objectives/Timeline

FY 2022-2023

- Develop multiple cyanotoxin analytical method workflow compatible with extraction solvents
- Optimize extraction conditions for cyanotoxins method, and test isotopic standard

FY 2023-2024

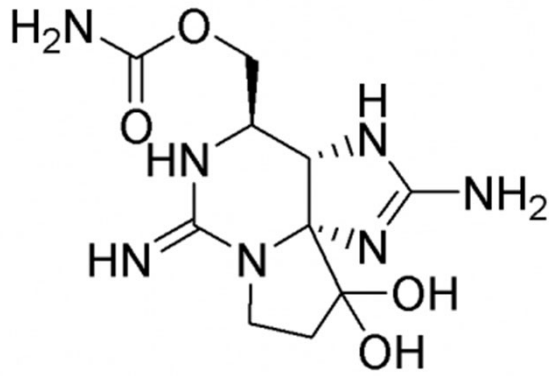
- Validate method performance in target matrices using spike/recovery studies
- Establish limits and publish method/standard operating procedure

FY 2025-Beyond

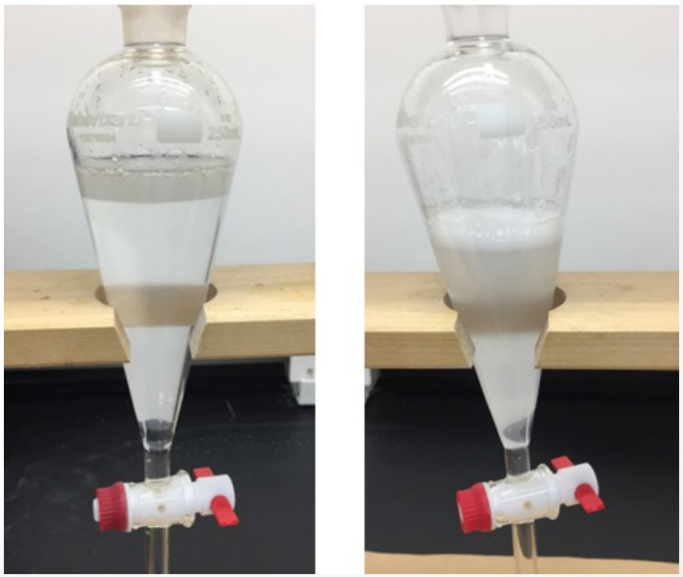
- Field testing of method in HAB-impacted sites and collaborations
- Internal use for ORD cyanotoxin/HAB research

Other Research Priorities

- New targets – Saxitoxin and related paralytic shellfish poisons (PSPs) are another important class of cyanotoxin, but extraction and stability are concerns.
- Comparing 'total' methods for toxins with many congeners, (e.g. MCs) with individual extraction methods
  - Ongoing EPA research on a 'total' microcystin method for tissue
- Recovery of protein-bound or metabolized toxins from tissue, and what they imply for health effects
- Where are toxins sequestered in tissue, is it species dependent?
  - Muscle, skin, fillet, liver, gut?
- Cross-validation across various tissues/species
  - Matrix interferences vary by species
- Exposure studies – Using cultured cyanobacteria and extracted toxins from culture to assess health effects/ingestion/depuration



Saxitoxin, which has many structurally similar PSPs in the environment



Extraction of a 10 mg fish sample (left) and 100 mg fish sample (right), showing lipid material interfering with extraction

Conclusions

- EPA/ORD research on cyanotoxin measurements in tissues is a long term research priority
- A multi-toxin LC-MS/MS workflow is the overall research object objective, ideally with a single, or two compatible extraction procedures
  - Significant work is needed to develop and validate the procedures for extracting, processing, and analyzing the multiple toxins for the method
- Isotope dilution techniques will be used where available to monitor and validate extraction and analysis performance
  - May be limited by availability of labeled standards
- Many ongoing and emerging research projects within EPA will be supported with this method

Photography by Jamie Mac Arthur

More Information

- EPA Cyanohabs Website:

https://www.epa.gov/cyanohabs

- EPA Methods for Cyanotoxins:

https://www.epa.gov/ground-water-and-drinking-water/detection-methods-cyanotoxins

- EPA Research Planning Website:

https://www.epa.gov/research/strategic-research-planning-epa

Thank you!

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