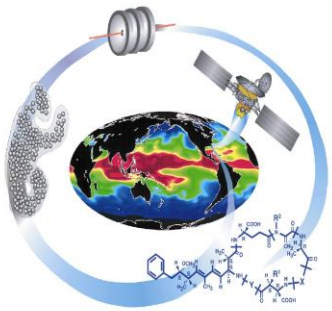
Final Report

Cyanotoxin Detection and Quantification and Instrumentation Workshop (CDQIW)



WORKSHOP
ON
Cyanotoxin Detection
and Quantitation
and Instrumentation

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Center for Ocean Technology
College of Marine Science- USF

Preamble

1.1 **Definition Activity**

Title: Cyanotoxin Detection and Quantification and Instrumentation Workshop (CDQIW)

Purpose: To develop instrumentation and probe/sensors to detect blue-green/cyanobacterial species and toxins

Goal: To develop and inline, realtime detector for toxic cyanos presence and amount. To be used as field devices and in-situ devices.

The purpose of the CDQIW Workshop was to focus perspective onto the status of new instrumentation for cyanotoxin detection in the field that has broad applicability to freshwater and ocean science research projects, regulatory activities and early warning and monitoring activity. Interactive activities between scientists, technologists, and regulatory personnel were deemed necessary to evolve the level of description of autonomous equipment as applied toward observational, experimental, and analytical water quality programs and the water sciences. The targeted technology areas included data collecting and observational systems, data communication based field devices, long-term sensors, and in-line underwater vessels. Considered was the opportunity to adapt technology or methodologies from other fields (e.g. clinical) or for modifying existing instrumentation for water monitoring purposes based on workshop recommendations. The workshop was specified as a venue to assess current status of the technology and instrumentation and to recommend areas for future development. Prior to the meeting, the pre-planning participants concluded that there was a need to develop an integrated system of monitoring for supplying real-time information to decision makers and to supply data to mapping and modeling efforts. The preferred embodiment of a field instrument was defined as one that would both verify the toxin and the toxigenic organism. A coupling of mass spectrometery or antibody detection with genetic probes was the agreed upon analytical strategy. Automation technologies were determined to be mature and would be inserted after the analytical issues were resolved.

Narrative

Detecting microbial contamination in the field is a demanding challenge for scientists and regulatory personnel. There are no clear technologies available, nor guidelines to address the extent of the problem of field microbial contamination and detection. In order to provide contamination information for the management of the field microbial hazards, the monitoring program needs to understand the advances in technology at its current state and the predicted technologies of the future. The goal of the workshop was to provide an assessment of the state of the technical art from subject matter experts within the cyanobacteria microbial community and to develop a roadmap towards a research sub-discipline of autonomous environmental monitoring instrumentation for cyanobacterial contamination in the field.

The topics of highest importance included:

- Techniques in the lab amenable to field monitoring,
- Validation and quality control of the field data,
- Validation of methods for detection,
- Development of field monitoring programs for real-time monitoring,
- Latest regulatory contaminant candidates and future ones
- Latest EPA method evaluations and expectations
- Surveys of new microbial field monitoring techniques.
- New techniques precision levels and accuracy, cost, real-time capability and ruggedness.

The session's goal was to survey the new methods, strengths and weaknesses of the various new methods, developing practical suggestions for an implentation plan for the new techniques and whether a permanent site of information was needed to support individuals interested in the issues of cyanobacterial presence in the environment.

1.2 Workshop

1.2.1 Outline of Workshop Program

Agenda

Monday August 20, 2001

Registration and Continental Breakfast: 8:00AM – 8:30

Technical Session I: 8:30AM-12:30 (Bayboro Room)

8:30AM- **Introduction and Need for Cyanotoxin Detection and Automation Workshop** (Karen Steidinger - FMRI)

9:00AM- **A Review of Detection Methods for Cyanotoxins** (Wayne Carmichael - Wright State University)

9:30AM- **Current and Emerging Techniques used to Identify Harmful Cyanobacteria** (Greg Boyer – State University of New York)

Break- Coffee

10:30AM- **Ecological and Molecular Characterization of Toxic Cyanobacterial Blooms** (Hans Paerl - University of North Carolina)

11:00AM- **Cyanotoxins in Florida's Surface Waters- Implications for Drinking Water Supplies** (John Burns - Cyanolab)

11:30AM- **EPA Regulatory Process and Monitoring for Unregulated Microbial Contaminants** (James Sinclair – EPA)

12:00PM- **Water Treatment Plant Operational Impacts from Cyanobacteria** (Bruce Macleod – Manatee County Water Quality)

12:30 – 1:30PM Lunch (On Your Own)

1:30 – 5:30PM **Field Trip**: Tour of Water Treatment Process Facility- Manatee County

Monday Evening Free

Tuesday August 21, 2001

Continental Breakfast: 8:00AM – 8:30

Technical Session II : 8:30AM-11:30 (Bayboro Room)

8:30AM- **ESP- An Experimental Platform for Remote Detection of Microorganisms and Substances They Produce** (Chris Scholin – MBARI)

9:00AM- **Assays for Algal Toxins: Some Considerations for Remote Detection** (Greg Doucette – NOAA)

9:30AM- **Development of the ESP Prototype- An Engineering Perspective** (Gene Massion- MBARI)

Break - Coffee

10:30AM- **Algal Monitoring on the Ohio River** (Judy Westrick- Lake Superior State University)

11:00AM- **Issues in Extraction, Concentration, and Analysis of Target Analytes from Water Systems- Automated Solid Phase Extraction** (K.C. Van Horne – ChromaKnowledge)

11:30AM- **Microsystems Technology for Cyanobacterial Bio/Chemical Detection- Useful Technology Paradigm?** (David Fries – University of South Florida)

12:00 – 1:00PM Lunch (On Your Own)

1:00 – 5:30PM - **Working group discussions: Science, Regulatory, Technology**

Topics

- Summarize the Science Base, Regulatory Base, Technology Base

- Needs from the resource management and regulatory perspectives.
- What do we need to do now and in the future?
- What are the logistical constraints of meeting those needs?
- Create Initial Roadmaps: Science, Technology, Regulations

Break

Topics

- Outline 'requirements document'
- What do we need to measure exactly/how often/where?
- What are the logistical constraints associated with those measurements?
- What are the primary cost constraints?
- Regulations: over the horizon
- What technology is available now that best supports our needs?
- Is there a need to develop new technology to meet our needs, and if so what specifically is desired/required in the near and long term?

Break - Refreshments

Topics

- Science/Engineering synthesis – towards a plan of action
- Above synthesis with Regulatory Horizon
- Given the needs and constraints identified above, how can we best respond immediately and in the near (5 year horizon) future?
- Draft preliminary Roadmap: prioritize goals and objectives.
- What steps should be taken to implement that plan?

Workshop Dinner: 6:30 until (**Ovo Café** 555 Central Avenue-Downtown St. Pete)

Wednesday August 22, 2001

Continental Breakfast: 8:00AM – 8:30

8:30 – 12:00PM - **Working group discussions: Final Summaries and Roadmap (Bayboro Room)**

Topics

- Summaries of group discussions

- Synthesis of how we proceed – toward a workshop report and roadmap creation

- List of Action Items- Assignments, Responsibilities

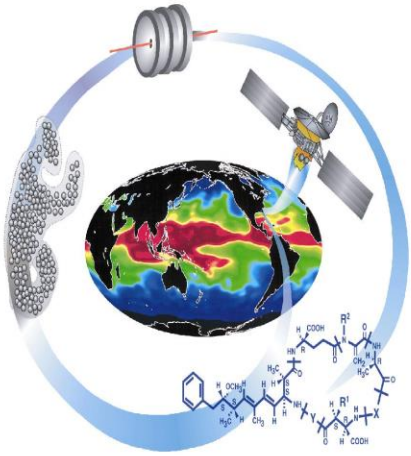
*Break-Coffee

Topics

- Synthesis of recommendations and plan for action items
    - Science
    - Regulations
    - Technology
    - Publish Workshop Report in Journal
    - Follow on Funding
    - Future Workshop

- Workshop ends

Lunch 12:00 – 1:30PM. (Lunch Provided - Hilton)



WORKSHOP
ON
Cyanotoxin Detection and Quantitation and Instrumentation

CENTER FOR OCEAN TECHNOLOGY

Presented by

University of South Florida

USF FMRI

August 20-22, 2001

St. Petersburg, FL

Downtown St. Petersburg Hilton

1st St. South and 4th Ave. South

Results

1.2.2 Scope out Problem of Cyanotoxins in the Environment/Industry

The primary and immediate need identified was to define the scope of the cyanotoxin hazard problem. It was recognized by the participants that the answer would require multi-agency cooperation to set the framework for the extent of the presence of contamination, R&D, treatment options, potential regulation, and event response driven planning/bloom mitigation. The fundamental question asked was: **is there a problem and what is the proper response**? It was concluded that **without the real-time instruments it is impossible to certify the true extent of the problem**. The lack of detail on the magnitude of the problem made evident the need for the advanced monitoring technology and the need for the workshop. The participants agreed that the environmental presence and extent of hazard is a very complex issue. Complex problems associated with multiple toxins/organisms that may or may not produce toxins verified the requirement for multi-parameter detection and real-time detection to characterize the ecological dynamics. Ultimately, establishment of analytical guidelines was deemed desirable. What should be measured, how and when? What levels of toxins/organisms were of concern, and the importance of recognizing the site-specific nature of the problem. Issues such as understanding the acute health implications of cyano blooms, mitigation/treatment options and the importance of addressing the health risks of chronic, low-dose exposure to cyanotoxins was noted, but recognized to be outside the scope of the workshop.

A further conclusion from the definition activity was that all issues of organism and toxin detection must be placed in the context of **action/alert levels**, of which only one exists in the U.S. at the present time (Oregon/microcystin). It is these levels that should drive development of detection methodologies in terms of sensitivity/limits of detection requirements. In the absence of these values, action/alert levels for cyanobacteria and cyanotoxins adopted by other countries should serve as guidelines and provide a framework for development of advanced detection capabilities.

1.2.3 Identify what can be accomplished with existing technology.

To address the need for real time detection a primary question was posed: what technology, methods, or probes existed for both toxins and toxigenic organisms and when and how to deploy that technology? The question was asked from a perspective of the specific needs for the drinking water industry and the goal was to recommend demonstratable, existing and simple methods of analysis.

When and how:

Spatial requirements

Recognized three zones/areas targeted for monitoring of organisms and/or toxins

Water treatment plant: toxins only

- optimally, would like to have ability to follow toxin level throughout all treatment stages to assess efficacy of toxin removal for each step (intake, first stage, second stage, etc.)

Source water: organisms and toxins

- monitoring at this stage will provide early warning of possible bloom/toxicity event, allowing increased reaction time

Watershed: organisms and toxins

- understand the dynamics of the ecology of cyanobacteria

Temporal requirements

Water treatment plant: high frequency: multiple times/day

Source water: moderate frequency: daily to multiple times/week

Watershed: required resolution is project driven

Note: cyanobacteria/cyanotoxin issues were recognized in the context of recreational water usage, but were considered outside the scope of the present initiative.

Toxins

Recommended strategy is to identify a site-specific subset of cyanobacterial toxins that are most relevant to a location

(e.g., microcystin LR, YR, RR, RA, anatoxin-a, cylindrospermopsin)

- note: need standards and antibodies for suite of toxins

Need to account for both particulate and dissolved forms of toxin;

-even if no toxin in water, but organisms are present then should disrupt cells to assess potential toxin load...the cells could leak or lyse later.

Confirm then Screen detection strategy:

-Need to adopt a two-tiered approach to toxin detection consisting of a rapid, reliable screening of samples, followed by confirmation by analytical methods

Most immediate need that fulfills the screen and confirm strategy is a **commercially available ELISA, which can be confirmed by LC-MS/MS**

- ELISA's antibodies should be selected to detect site-specific subset of toxins
- ELISA format should be user-friendly and not require a high level of technical expertise (e.g., dipstick outside of lab; multi-well, colorimetric/fluorometric in lab)
- Only ELISA kits for microcystin are available; however, all are not fully characterized in terms of cross-reactivity with various MC congeners
- Mass Spectrometry method developments and instrumentation configurations are evolving at a fast pace due to the availability of new ion sources and cheaper analyzers. As field portable instrumentation evolves, the possibility of achieving in-field real-time confirmation becomes a reality.

Toxigenic Organisms

Methods identified and to be considered were:

- nucleic acid probes
- diagnostic/signature pigments (compounds and gene)
    - potential for interference by autofluorescing substances
- combinations of the above two
- direct imaging (currently 50 um resolution)
- functional genes (e.g. *nif*; but, note selectivity)

Of the technologies currently available**, nucleic acid probe-based detection of the organisms has the most promise,** simplicity and likelihood of success in the short term.

Important to note that organism and chemical detection is also needed in the context of taste and odor issues and that the same cyanobacterial species may be potential toxin producers; efforts aimed at organism and chemical detection may thus address both needs.

Summary of the identified analytical strategies for toxin and toxigenic organism:

Available/Identified Technology-Toxin	**Available/Identified Technology-Organism**
Antibody based diagnostics-	Direct Imaging
HPLC-fluorimetry	Genetic Probes
HPLC-MS	Antibody Probes
HPLC-Fluorimetry-MS	Pigment Analysis- chl-A

Note: microcystin, cylindrospermosin, anatoxin antibodies for all three and standards needed. Mass spectrometry is advantageous but may be still focused on research environment and not on water process control.

1.2.4 Identify what could be accomplished beyond today's technology

General analytical strategy recommended:

Establish bench-top methods for detecting toxin/organisms first, then consider automation.

Also identified as necessary for evolution of the technology:

-Must standardize methods to allow comparability of results among the user. Need to validate certain methods (e.g., AOAC collaborative trials); in context of action/alert levels.

-Automation of detection capabilities

(*Limiting factor:* development and testing of protocols for specific applications)

- Emphasize need to make instruments available for method development

-Communication of data and information-knowledge management

Establish a network of user groups and convene regular meetings to maintain linkages and communication on timely issues.

Develop multi-purpose website aimed at the needs of specific target groups

- User group (i.e., water treatment plants, water districts, etc.)/no public access
    - Species ID pictures/information
    - Information clearinghouse (e.g., meeting notification, communication of research findings)
    - Links to related sites
    - Data repository

1.2.5 Automated Instrument Design Discussion

Engineering requirements for autonomous/remote systems are considered to be in place and will require minimal modifications to accommodate individual testing protocols. Sample prep/protocol automation is a case specific technology, so sample modulation processes for targeted toxins and toxigenic organisms will have to be individually developed. However, the available sample preparation procedures are multiplying in the commercial field and literature due to the emerging area of field analytical detection. Telemetey of data from remote sites was also recognized as an enabling technology. The recommendation was to utilize the huge advances in wireless technology both local are and wide area and the increasing availability of long range communications via satellite

phone such as the Orbcomm or Iridium low earth orbiting satellite systems now available. Inexpensive, long-lived, rugged power sources for the remote, autonomous detectors was mentioned but the issue was raised as a universal problem existing for all remote instruments and thus the resolution will have to come from the every changing state of the art in battery technology. Since the strategy was to establish bench-top methods for detecting toxin/organisms first, then consider automation, further discussions and recommendations about automation design were extended into the future where the technology at post bench-top method will be most certainly more advanced than current levels.

1.2.6 Proof of Concept –Design of Experiments

Four recommendations for experiments (and there status as of October) were promoted:

1.**Establish and maintain culture standards**

(Wright State University- culture initiated and in process)

2. **Establish Reference standards -toxins**

(Wright State U – coupled to microcystis production as a first step)

3. **Couple existing automated instruments**- genosensor (ESP)-(MBARI) and mass spectrometer (UMS)-(USF) into a design approach for a generic technology platform for toxin and toxigenic organism screening and confirmation.

(USF- Manatee County WTP: meetings and site visits finished for real time MS detection effort for taste and odor chemicals as a first step toward toxin detection)

(MBARI- 2nd generation ESP being considered and designed, which will serve as prototype field unit for gene screening)

4. **Show proof of concept of direct imaging, counting and analysis using imaging techniques.**

(USF- sub 50 uM imager conceptualized; commercial instrument FlowCAM , flow cytometer/camera/microscope combination being evaluated for measurement capabilities concurrently against USF specific instrument, microchip based version also designed).

1.2.7 Roadmap to Implementation/Next Steps

Summarized are the recommended next steps/ targets and their timeframes as provided by the workshop participants and agreed upon:

B group results

Slide 1-B group

Regulatory Context for Detection Requirements

All issues of organism and toxin detection must be placed in the context of **action/alert levels**, of which only one exists in the U.S. at the present time (Oregon/microcystin).

It is these levels that should drive development of detection methodologies in terms of sensitivity/limits of detection requirements.

In the absence of these values, action/alert levels for cyanobacteria and cyanotoxins adopted by other countries should serve as guidelines and provide a framework for development of detection capabilities.

The importance of addressing the health risks of chronic, low-dose exposure to cyanotoxins was noted, but recognized to be outside the scope of our current focus on acute, episodic events.

Slide 2-B Group

Monitoring Needs Specific for Drinking Water

Spatial requirements

Recognize three zones/areas targeted for monitoring of organisms and/or toxins

Water treatment plant: toxins only

- optimally, would like to have ability to follow toxin level throughout all treatment stages to assess efficacy of toxin removal for each step (intake, first stage, second stage, etc.)

Source water: organisms and toxins

- monitoring at this stage will provide early warning of possible bloom/toxicity event, allowing increased reaction time

Watershed: organisms and toxins

Temporal requirements

Water treatment plant: high frequency…multiple times/day

Source water: moderate frequency…daily to multiple times/week

Watershed: required resolution is project driven

Note: cyanobacteria/cyanotoxin issues were recognized in the context of recreational water usage, but were considered outside the scope of the present initiative.

Slide 3-B Group

Toxins

Identify a site-specific subset of cyanobacterial toxins that are most relevant to a location (e.g., microcystin LR, YR, RR, RA, anatoxin-a, cylindrospermopsin); develop standards & antibodies

Need to account for both particulate and dissolved forms of toxin; even if no toxin in water, but organisms are present then should disrupt cells to assess potential toxin load...the cells could leak or lyse later.

Need to adopt a two-tiered approach to toxin detection consisting of a rapid, reliable screening of samples, followed by confirmation by analytical methods

Most ***immediate need*** is for a commercially available ELISA, which can be confirmed by LC-MS/MS

- ELISA's antibodies should be selected to detect site-specific subset of toxins
- ELISA format should be user-friendly and not require a high level of technical expertise (e.g., dipstick outside of lab; multi-well, colorimetric/fluorometric in lab)
- Only ELISA kits for microcystin are available; however, all are not fully characterized

Intermediate term need would involve automated remote detection of toxins, with same selectivity, reliability, and temporal resolution as targeted ELISAs

Slide 4-B Group

Organisms

Methods to be considered:

- nucleic acid probes
- diagnostic/signature pigments (compounds and gene)
  - potential for interference by autofluorescing substances
- direct imaging (currently 50 m resolution)
- functional genes (e.g. *nif*; but, note selectivity)

Of the technologies currently available, nucleic acid probe-based detection of the organisms has the most promise and likelihood of success in the short term.

Important to note that organism detection is also needed in the context of taste and odor issues and that the same cyanobacterial species may be potential toxin producers; efforts aimed at organism detection may thus address both needs.

General Considerations

Must standardize methods to allow comparability of results among the users

Need to validate certain methods (e.g., AOAC collaborative trials); again, would like to do this in context of action/alert levels.

Slide 5-B Group

Automation of detection capabilities

Limiting factor: development and testing of protocols for specific applications

emphasized need to make instruments available for method development

Engineering requirements for autonomous/remote systems are considered to be in place and will require minimal modifications to accommodate individual testing protocols.

Communication of data and information

Establish a network of user groups and convene regular meetings to maintain linkages and communication on timely issues.

Develop multi-purpose website aimed at the needs of specific target groups

- User groups (i.e., water treatment plants, water districts, etc.)/no public access
  - Species ID pictures/information
  - Information clearinghouse (e.g., meeting notification, communication of research findings)
  - Links to related sites
- General public
  - Educational information

Slide 6-B Group

Identification of potential funding sources

- **F**ederal
- NOAA (e.g., Ocean Exploration)
- EPA
- Healthy Beaches
- AWARF
- Water Resources
- Piggyback with efforts addressing taste and odor issues
- Collaborations with international colleagues (e.g., Australia, EU, etc.)

TAKE HOME POINTS

- Define action/alert levels for organisms and toxins
- Develop, characterize, standardize, and validate rapid screening tests for organisms (nucleic acid probes) and toxins (ELISA)
- Transfer protocols for rapid screening tests to automated, remote platforms by providing access to lab-based instrument configurations
- Develop website to communicate data and information to users and general public
- Identify potential sources of support at federal, state, local levels and seek funding through collaborations among scientists and users of detection technologies

Slide 7-B Group

THE HOLY GRAIL

ALL CHEMISTRY/BIOCHEMISTRY ALL THE TIME

ULTIMATE GOAL IS THE CONCURRENT, CONTINUOUS DETECTION OF BOTH ORGANISMS AND TOXINS ON AN AUTONOMOUS, REMOTE PLATFORM IN REAL TIME

A-Group results

Slide 1-A Group

Cyanotoxin Workshop Breakout A'

- Considered discussion topics from the perspective of establishing priorities
  - Immediate needs
  - Looking ahead towards establishing R&D priorities and goals
    - 2 years
    - 2-5 years

Slide 2-A Group

Immediate Needs: Framing the Issue

- Detection of the organisms and toxins
  - Multiple suites of toxins
  - Organisms may or may not produce toxins
    - Genetic variation, physiological induction/suppression
  - Establishment of analytical guidelines is desirable
    - What should be measured, how and when?
    - What levels of toxins/organisms are of concern?
    - Important to recognize the site-specific nature of the problem
- Issues such as understanding health implications of cyano blooms, mitigation/treatment options, etc., are beyond the scope of this workshop
- Requires multi-agency cooperation

Slide 3-A Group

Immediate Needs: Define the Scope of the Problem

- Detection of Toxins
  - Need analytical standards
  - Establish methods that allow for inter-lab comparisons
  - Focus on bench-top sample processing
  - Employ existing ELISA (could be wide spread)
  - LC/MS (limited; some utilities and research groups)
- Detection of Organisms
  - Traditional microscopy/pigment analysis
  - Molecular probes where available
  - Compare with presence/absence of toxins where appropriate
- Focus on bench-top methods for detecting toxin/organisms first, then consider automation

Slide 4-A Group

Research Priorities and Goals: 2 Year Horizon

Toxins (Microcystins, Cylindrospermopsin, Anatoxin-a):

- Necessary reference standards widely available
- Establish standard extraction/detection methodologies
- Deploy/develop “dipstick” or ELISA kits
  - Promote simple tests that can be disseminated widely
- Merge antibody development with SPE-LC/MS technology
- Establish suite of sample collection sites/routine survey protocols
- Prototype automated systems
  - primary focus lab-based)

Slide 5-A Group

Research Priorities and Goals: 2 Year Horizon

Organisms:

- Establish and maintain culture standards
- Characterize organisms:
  - Morphology/pigment/physiology
  - Molecular sequence data (rDNA, Nif, etc.)
  - Toxin production (culture and natll samples may differ)
  - Work to identify genetic determinant of toxicity
- Develop / deploy "dipstick" detection methods
  - Promote simple tests that can be disseminated widely
- Establish suite of sample collection sites/routine survey protocols
  - Microscopy/probes
- Prototype automated systems

Slide 6-A Group

Research Priorities and Goals: 2-5 years

Utility Perspective:

- Better understanding of the problem scope and mitigation options
- Widespread distribution of "dipstick assays" for species/toxins
- Bench-top, in-line, automated systems for toxin detection

Slide 7-A Group

Research Priorities and Goals: 2-5 years

Watershed Management Perspective

- Better understanding of factors modulating cyano outbreak
  - Physical/chemical (temperature, salinity, nutrients, irridance)
  - Geographic distribution of toxic/nontoxic species or strains
    - Employ probes
    - Community structure (specific combinations of species)
- Remote organism/toxin detection (targeted sites)
- Widespread distribution of "dipstick" assays for species/toxins
- Evaluate cost/benefit of detection strategies
  - manual, automated; lab, *in situ*

The conclusion from the workshop for defining a device and the defining the field analytical research strategy for Cyanotoxin Detection and Quantification and Instrumentation was best be summarized by the B-group:

THE HOLY GRAIL

ALL CHEMISTRY/BIOCHEMISTRY ALL THE TIME

ULTIMATE GOAL IS THE CONCURRENT, CONTINUOUS DETECTION OF BOTH ORGANISMS AND TOXINS ON AN AUTONOMOUS, REMOTE PLATFORM IN REAL TIME

1.2.8 **CDQIW- Scope of Work Deliverable Activities**

List of Advances/Activities to Support Project

a. Established a CyanoWorking Group (CWG)/Identified participants (see table below)
b. Arranged for contacts between working group members
c. Identified source of culturing of purified Cylindrospermopsis toxin (Wayne Carmichael)
d. Identified source of production for microcystin toxins (Wayne Carmichael)
e. Arranged for subcontract to Wayne State University (Dr. Wayne Carmichael) to culture *Cylindrospermopsis raciborskii* for producing purified standard
f. Initiated and Completed Workshop Specification, Timing and Content (see Workshop announcement)
g. Initiated/Build Database of technologies for workshop critique and expansion (in-process: currently 15 potential competitive technical strategies identified)
h. Secured Tour of Water Treatment Plant for end user fact-finding effort.
i. Conduct a Workshop on the Topic of: Cyanotoxin Detection and Quantification and Instrumentation
j. Established Web Site template and content for Web Page for the workshop and for future portal for cyanotoxin detection technologies
k. Generated Prototype licensure agreement for any prototype technology requiring such that is generated from funded proof of concept experiments.
l. Initiated working document for workshop participants
m. Compensated collaborators and workshop participants for expenses related to the workshop
n. Advanced four experimental activities (final results in process)
o. Identified potential funding sources for continuation of project and development of a program on in-field automation and detection
p. Submitted reports on status and results from workshop and related activities.
q. Search (in process) for journal to create special topic issue on: **Cyanotoxin Detection and Quantification and Instrumentation**

Working Group Composition

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End of report