This project is being funded through the Focus Area Program, which enables WRF to solve broadly relevant subscriber issues and challenges with a targeted, sustained research effort. The program is developed around research Focus Areas: a topic area that is of high interest and priority to WRF subscribers because of a challenge or opportunity that is present, emerging, or anticipated, and for which research will help subscribers manage and address the challenge or optimize the opportunity. A focus area includes a discrete challenge or opportunity statement, measurable objectives, and one or more projects that will lead to applied solutions and benefits for WRF subscribers within a specified, relevant time frame.

This project is funded under the Focus Area titled, ***Cyanobacterial Blooms and Cyanotoxins: Monitoring, Control, and Communication Strategies*** *and is intended to support the Focus Area objective(s):*

- ***optimized watershed and source water management strategies***
- ***cost–effective control options***
- ***practical guidance for control and treatment***
- ***public outreach and communication strategies and tools***

FOCUS AREA RFP

Refinement and Standardization of Cyanotoxin Analytical Techniques for Drinking Water (RFP #4716)

Project Objective

The objective of this project is to evaluate existing chemical and biological methods for the analysis of cyanotoxins at low part-per-trillion (ppt) detection levels in raw and finished drinking water and provide unified methods and practical analytical guidelines for utilities to improve the quality of monitoring efforts.

Budget

Proposals may request WRF funds in the range of $375,000 - $416,000. WRF funds requested and total project value will be a criteria considered in the proposal selection process.

Background

In 2015, the United States Environmental Protection Agency (USEPA) published Health Advisory Levels (HALs) for states and utilities to protect the public from cyanotoxins in drinking water. The health advisory was set at 0.3 µg/L for microcystins (MCs) and 0.7 µg/L for cylindrospermopsin, which are not to be exceeded in drinking water for children younger than school age. Although not enforceable, the public health advisory is intended to trigger utility actions including increased monitoring, development of treatment strategies, and public notification of “do not drink” advisories. USEPA is also providing recommendations on how utilities can monitor and treat water for cyanotoxins. Additionally, the EPA has released a pre-publication copy of its proposed Fourth Unregulated Contaminant Monitoring Rule (UCMR4) which includes both individual cyanotoxins such as cylindrospermopsin, anatoxin-A, microcystin-LR and nodularin, and also “total” MCs in aggregate.

Several analytical techniques exist for the analysis of cyanotoxins. The most commonly used methods include bioassays such as enzyme linked immunosorbent assays (ELISA) and instrumental methods such as liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). These methods are sensitive and can detect nanogram/liter (ng/l) concentrations of cyanotoxins. However, they have different advantages and disadvantages and they may not always be appropriate in all situations and uses.

LC/MS/MS techniques can reliably identify and quantify toxins in water. However, they require expensive hardware and typically involve complicated sample preparation procedures, with correspondingly long turn-around times, making them unsuited for rapid screening and decision making. In addition, existing LC/MS/MS methods, such as the USEPA Method 544, are limited to only a subset of cyanotoxins for which commercial standards are available. In the case of MCs, fewer than 20 of over 150 known toxin congeners have purified analytical standards available. Similar complications exist for saxitoxin and related paralytic shellfish poison (PSP) toxins, which include a complex variety of structurally similar chemical species. These make LC/MS/MS methods less appealing as a primary screening tool, as they cannot conclusively prove the absence of PSPs or microcystins in a water.

The USEPA recommends using (ELISA) test kits for initial sampling/screening for "total" MCs, as described in USEPA Method 546. The key advantages of ELISA-based techniques are ease of use and lower cost: Analyzing ELISA results does not require instrumentation beyond a relatively inexpensive plate-reader, and can be readily employed for on-site screening at water utilities. In addition, ELISA methods for quantifying MCs are broadly selective for almost all known congeners, albeit with varying responses, which makes it more suitable as a screening tool. However, ELISA results are only semi-quantitative and potentially subject to interference from the water matrix, congener composition, and other parameters potentially leading to false positives or negatives. In addition, the detection limit of ELISA for MCs is typically around 0.3 µ/L, equal to the health advisory's threshold. Accordingly, in the case of MCs any positive detection could result in exceedance of the health advisory limit, placing a financial burden on utilities and causing loss of public confidence in the safety of drinking water.

At present, there is a great deal of uncertainty relating to the state of the art for screening and confirmation of cyanotoxin samples. A great deal of discussion has taken place regarding microcystins in particular owing to their role in Toledo, but there are still unanswered questions about cross-reactivity and specificity of the assay. For cylindrospermopsin and anatoxin-A, there are well established LC/MS/MS methods (Such as USEPA Method 545) for measuring samples, but a dearth of studies comparing and contrasting sample results with ELISA, which is commonly used by utilities. Detection and measurement of saxitoxin and other PSP toxins is complicated by their potential interconversion and sensitivity to sample preparation techniques. Water utilities need robust and dependable methods in order to monitor cyanotoxins in the source water, through the treatment process, and at the tap as well as to make appropriate decisions regarding operations and public notifications.

In recent years, a large number of research efforts have been dedicated to cyanotoxin methods. This project will build upon on on-going WRF Project 4647 "Performance Evaluation of Methods for the Analysis of Cyanotoxins" (expected to be completed in Summer 2017). The goals of that project are to identify and compare the strengths and weaknesses of the Ohio EPA ADDA-ELISA method and EPA Method 544, *Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).* The method comparison specifically focuses on the ability of each method to successfully quantify, with precision and accuracy, microcystins (MC) at part-per-trillion detection levels in drinking water and source water. The resulting information obtained during that project can be used to design a larger and more comprehensive inter-laboratory study to compare and evaluate the methodologies and variability between methods and laboratories for cyanotoxins including MCs and nodularin, cylindrospermopsin, anatoxin-A, saxitoxin and other PSPs.

Research Approach

Because of the continuing advancement in analytical methods for cyanotoxins and to avoid redundancy it is expected that the proposing team conduct a thorough review of the literature.

The approach to this project should include:

- Rigorous comparison of testing procedures
- Standardization of steps for raw and finished water samples
    - Sample collection and preservation
    - Extraction
    - Sample preparation
    - Analysis
- Evaluation of reporting limits in the context of human health guidelines. Propose refinements to existing methods to improve detection limits
- Interlab comparison of existing methods and new standard procedures
- At a minimum microcystin and cylindrospermopsin should be investigated, but consideration should also be given to saxitoxin and other PSP toxins, nodularin, anatoxin-a, etc.

The final product of this project will consist of a set of standardized methods with associated quality assurance protocols. In addition, the report is expected to provide guidance to help utilities determine the most appropriate use of the methods for identification and verification of toxin measurements in water.

Proposal Preparation Instructions

Proposals submitted in response to this RFP must be prepared in accordance with the Water Research Foundation document "Guidelines for Focus Area Program Proposals." The most current version of these guidelines is available at http://www.waterrf.org/funding/Pages/proposal-guidelines.aspx . The guidelines contain instructions for the technical aspects, financial statements and administrative requirements that the applicant must follow when preparing a proposal.

Eligibility to Submit Proposals

This RFP solicits proposals from all technically qualified U.S. based or non-U.S. based applicants, including educational institutions, research organizations, federal or state agencies, local municipalities, and consultants or other for-profit entities.

WRF's Board of Trustees has established a Timeliness Policy that addresses researcher adherence to project schedule. The policy can be reviewed at http://www.waterrf.org/funding/Pages/policies.aspx. Researchers who are late on any ongoing WRF-sponsored studies without an approved no-cost extension are not eligible to be a named participant in any proposal. If you have any questions about your eligibility for WRF projects, please contact the WRF Research Manager listed at the bottom of the RFP.

Administrative, Cost and Audit Standards

WRF's standards for administrative, cost and audit compliance are based upon and comply with Office of Management and Budget (OMB) Uniform Grants Guidance (UGG), 2 CFR Part 200 Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards, and 48 CFR 31.2 Contracts with Commercial Organizations. These standards are referenced in the WRF's "Guidelines for Focus Area Program Proposals" and include specific guidelines outlining the requirements for Indirect Cost Negotiation Agreements, Financial Statements and the Statement of Direct Labor, Fringe Benefits and General Overhead. Inclusion of indirect costs must be substantiated by a negotiated agreement or appropriate Statement of Direct Labor, Fringe Benefits and General Overhead. Well in advance of preparing the proposal, your financial staff should review the detailed instructions included in WRF's annually released "Guidelines for Focus Area Program Proposals."

Budget and Funding Information

The funding available from WRF for this project is in the range of $375,000 - $416,000. A minimum 25 percent of the total project value must be contributed by the applicant (i.e. the applicant's minimum contribution must equal one-third of WRF funds requested). Acceptable forms of applicant contribution include cost-share, applicant in-kind or third-party in-kind that comply with 2 CFR Part 200.306 Cost sharing or matching. The applicant may elect to contribute more than 25 percent to the project but the maximum WRF funding available remains fixed at $416,000. **Proposals that do not meet the minimum 25 percent of the total project value will not be accepted.**

Period of Performance

The proposed project schedule should be realistic, allowing ample time for the preparation of final reports and for review of project results. It is WRF's policy to negotiate a reasonable schedule for each research project. Once this schedule is established, WRF and its sub-recipients have a contractual obligation to adhere to the agreed-upon schedule. Under WRF's No-Cost Extension Policy, a project schedule cannot be extended more than nine months beyond the original contracted schedule, regardless of the number of extensions granted. The policy can be reviewed at http://www.waterrf.org/funding/Pages/policies.aspx.

Utility and Organization Participation

WRF is especially interested in receiving proposals which include both participation and contribution of resources from water utilities and organizations in the research effort. Information on utilities and/or organizations that have indicated an interest in participating in this research project is attached. While WRF makes utility and organization participation volunteers known to applicants, it is the applicant's responsibility to negotiate utility and organization participation in their particular proposal, and the utilities and/or organizations are under no obligation to participate.

Application Procedure and Deadline

Proposals are now being accepted exclusively online in PDF only format and must be fully submitted before November 1, 2017, 5pm Mountain Time. All the forms and components of the proposal are available online in the "Proposal Component Packet" zip file. A login is required to download this packet

and use the proposal website. This information is available at
https://proposals.waterrf.org/Pages/RFPs.aspx
The online proposal system allows submission of your documents until the date and time stated in the RFP. To avoid the risk of the system closing before you press the submit button, do not wait until the last minute to complete your submission.

Questions to clarify the intent of this Request for Proposals and WRF's administrative, cost and financial requirements may be addressed to the Research Manager, Djanette Khiari, at 303.734.3478 or by e-mail at dkhiari@waterrf.org.

4716 UTILITY VOLUNTEERS

The following utilities have indicated an interest in possible participation in this research. This information is updated within 24 business hours when a utility submits a volunteer form and this RFP will be re-posted with the new information. **(Depending upon your settings, you may need to click refresh on your browser to load the latest file.)**

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