Laboratory Methods for the Study of Harmful Cyanobacteria Water blooms: "The Cyano-HABs"

Wayne W. Carmichael
Professor-Aquatic Biology/Toxicology

Department of Biological Sciences
Wright State University
Dayton, Ohio 45435 U.S.A.

Tel: 937-775-3173
Fax: 937-775-3320
E-mail: wayne.carmichael@wright.edu

University website address:
http://biology.wright.edu/faculty/carmichael/labhome.htm

Background

Increasingly, harmful algal blooms (HABs) are being reported worldwide due to several factors, primarily - eutrophication, climate change and more scientific investigation (Hallegraeff et al., 1995). HAB organisms include those causing: PSP (paralytic shellfish poisoning), DSP (diarrhetic shellfish poisoning), NSP (neurotoxic shellfish poisoning), ASP (amnesic shellfish poisoning) and CTP (cyanobacteria toxin poisoning). All but CTP organisms are mainly a marine occurrence. CTPs occur in freshwater lakes, ponds, rivers and reservoirs throughout the world. Organisms responsible include an estimated 40 genera but the main ones are Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nostoc and Oscillatoria (Planktothrix) (Carpenter and Carmichael, 1995). Cyanobacteria toxins (cyanotoxins) include cytotoxins and biotoxins with biotoxins being responsible for acute lethal, acute, chronic and sub-chronic poisonings of wild/domestic animals and humans. The biotoxins include the neurotoxins; anatoxin-a, anatoxin-a(s) and saxitoxins plus the hepatotoxins; microcystins, nodularins and cylindrospermopsin (Carmichael, 1997; 2001) (Table 1).

Table 1. Name and Producer Organism for the Biotoxic Cyanotoxins

NAME	PRODUCED BY
Neurotoxins	
Anatoxin-a Homo-Anatoxin-a	*Anabaena, Aphanizomenon, Oscillatoria (Planktothrix)*
Anatoxin-a(s)	*Anabaena, Oscillatoria (Planktothrix)*
Paralytic Shellfish Poisons	

(Saxitoxins)	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Planktothrix, Trichodesmium,*

Liver Toxins

Cylindrospermopsin	*Aphanizomenon, Cylindrospermopsis, Rhaphidiopsis, Umezakia*
Microcystins	*Anabaena, Aphanocapsa, Hapalosiphon, Microcystis, Nostoc, Oscillatoria (Planktothrix), Synechococcus*
Nodularins	*Nodularia* (brackish water)

Contact Irritant-Dermal Toxins

Debromoaplysiatoxin, Lyngbyatoxin	*Lyngbya* (marine)
Aplysiatoxin	*Schizothrix* (marine)

Concern for animal and human health impairments due to toxic cyanobacteria arises from several sources of information. The main one concerns reports of animal poisonings from ingestion of toxic water blooms beginning with the later part of the 1800's. It was not until the 1950's, with pioneering work of scientists in Canada, the United States, Australia and South Africa, that we began to understand that cyanobacteria could indeed produce highly toxic compounds. The animal and human intoxication reports were first summarized in the 1960's by Schwimmer and Schwimmer (1964, 1968). These reports have been updated by several works including Sivonen (1990), Carmichael (1992), Ressom, et al. (1994) and Yoo, et al. (1995). In addition, a major effort to compile all available information on toxic cyanobacteria including issues of human health, safe water practices, management, prevention and remediation has been published by the World Health Organization (Chorus and Bartram, 1999). This book is designed as a manual for water and health authorities in addressing all issues of toxic cyanobacteria that they may be confronted with.

Along with the WHO manual came a study on the risk assessment of cyanotoxins in drinking and bathing waters. Risk assessment of microcystins (as Microcystin-LR) indicate that a level of 1 $\mu g\ L^{-1}$ should be considered a guideline value for maximum allowable concentration (MAC) based upon an adult consumption of 2 L day^{-1}. Other MAC's for the neurotoxins and cylindrospermopsin will not be set until more basic toxicology and epidemiology are available. In addition, the State of Oregon in the U.S.A. has set a regulatory level of 1 $\mu g\ g^{-1}$ for microcystins (as Microcystin-LR) in human food supplements made from the filamentous cyanobacterium Aphanizomenon flos-aquae (AFA). In this case the AFA is not the producer of microcystin but because it is harvested from a natural lake (Klamath Lake), other cyanobacteria present as a normal part of the harvested algae (i.e., Microcystis aeruginosa a known producer of microcystins) are the source of this hepatotoxins. Health authorities from several countries are also evaluating the risks of cyanotoxins in algae and may also adopt guideline or regulatory levels for microcystins in drinking and recreational waters and algae food products.

Confirmation of human deaths from cyanotoxins is limited to exposure through renal dialysis. Microcystins were implicated as the major contributing factor in liver failure and death of at least 52 humans, in 1996, at a haemodialysis center in Caruaru, Brazil (Jochimsen, et al. 1998, Carmichael, et al. 2001). This tragic but avoidable event points to the importance of understanding cyanotoxins as health hazards in drinking waters and then communicating this information, including the correct identification of cyanobacteria responsible for poisonings, to water authorities and public health officials. Since most of the worlds reservoir and lake based water supplies are subject to increasing nutrient levels, it is probable that episodes of cyanotoxin poisoning will continue unless measures are taken to improve our understanding of their role in water-based diseases. For these reasons it is important to consider the prevention, management and mitigation of CyanoHABs.

Prevention, Management and Mitigation Strategies for Control of Cyanobacteria and Their Toxins

The first goal in the control of cyanobacteria water blooms in public water supplies should be the implementation of ways to prevent or at least minimize those conditions, which lead to their excessive growth. In addressing these growth requirements the two main problems are nutrients and light (Chorus and Bartram 1999-Ch. 8). Critical to nutrient and light control is proper management of the water supply watershed and the water reservoir. There are two main ways this can be done. They are termed 'top-down' and 'bottom-up control'. Top down control is also termed bio-manipulation and is the management of higher trophic organisms to maximize grazing of algae and thus reduce cyanobacteria abundance. The bottom-up approach is to restrict the supply of essential nutrients and thus restrict growth. The key action for nutrient control of cyanobacteria is reduction of external phosphorus and nitrogen. In this argument, bottom-up control is extended to include the restriction to supply of all resources. Therefore, the manipulation of the light climate by artificial mixing is also included in bottom-up control.

The management of cyanobacteria water blooms is best approached by the development of a risk-management framework. This framework should define the needed approaches for situation assessment, management actions (often referred to as the Alert Levels Framework), and appropriate planning and response (Chorus and Bartram 1999, Ch. 6-7).

The mitigation, or minimization, of the harmful effects of a cyanobacteria water bloom can be approached in three main ways (Chorus and Bartram 1999-Ch. 9). The first involves selecting the best abstraction method or location for bringing water into the treatment plant. This can be done by choosing an optimum position for off take, or by abstracting surface water through bank or ground filtration. The second major way to mitigate cyanobacteria water blooms is to choose the optimum water treatment technique(s) in the water treatment plant. These include the proper choice of coagulation, precipitation, sedimentation, filtration and adsorption technologies for water bloom management. The third and final way to mitigate cyanobacteria water blooms is with chemicals.

Chemical control of algae in water supply storages has been a widespread water quality mitigation practice for over 100 years. Copper sulphate has generally been the algaecide of choice and records of its use date from 1890 in Europe (Sawyer 1962), 1904 in the U.S. (Moore and Kellerman 1905), and since at least the mid-1940's in Australia (Burch et al. 2001).

An extensive survey of water utilities in the U.S. and Canada in the 1980's indicated that copper sulphate is by far the most widely used algaecide, although other alternatives are used under some circumstances (Casitas Municipal Water District 1987). A summary of the range of compounds that have been used as algaecides is given in Table 2.

TABLE 2: Common algaecides and their formulations (after Burch et al., 1998)

COMPOUND	FORMULATION
Copper sulphate	$CuSO_4.5H_2O$
Copper II alkanolamine complex	Cu Alkanolamine $.3H_2O^{++}$
Copper - ethylenediamine complex	$[Cu(H_2NCH_2CH_2NH_2)_2(H_2O)_2]^{++}$ SO_4
Copper - triethanolamine complex	Cu $N(CH_2CH_2OH)_3.H_2O$
Copper citrate	$Cu_3[(COOCH_2)_2C(OH)COO]_2$
Potassium permanganate	$KMnO_4$
Chlorine	Cl_2
Lime	$Ca(OH)_2$
Barley straw	

In summary, for mitigation of cyanobacterial water blooms the first priority should be removal of intact cells, using selected abstraction points followed by separation techniques such as coagulation or membrane filtration. Chemical treatment methods have and are being evaluated with chlorination and ozonation being effective for the destruction of residual dissolved microcystins and cylindrospermopsin at moderate water temperatures. Possible temperature effects on these oxidation reactions are currently unknown. Anatoxin-a can be effectively removed using ozone, although chlorine is relatively ineffective. Oxidation techniques do not appear to be the best method for the treatment of saxitoxins under normal treatment plant operating conditions. Powdered activated carbon can be effective for the removal of all toxins, except, perhaps, a few microcystins, provided the appropriate carbon, and the correct dose is applied. Granular activated carbon filters show a limited lifetime for the adsorption of most microcontaminants, including cyanotoxins. The biodegradation of cyanotoxins across GAC filters shows great potential as a treatment process.

Laboratory methods for the Study of Harmful Cyanobacteria Water blooms

In order to effectively study CyanoHABs, it is necessary to consider several distinct areas including:

- Ecology and Physiology of Cyanobacteria Water blooms
- Sampling, Identification (Taxonomy) and Quantification of Cells
- Culture and Handling of Toxic Cells
- Preparation and Processing of Cells, Tissues and Other Matrices for Toxin Analyses
- Cyanotoxin Detection and Analyses Methods

    - Biological – Bioassay

    - Biochemical - i.e. Immunological (ELISA); Enzyme (PPIA)

    - Analytical - HPLC, MS, NMR

    - Genetic – PCR and others

Laboratory studies focus on the latter four areas and our laboratory work has emphasized the last topic area-detection and analyses of cyanotoxins.

Over the past 30 years these studies have involved:

- Biological - Bioassay

    * Small animal - mouse, invertebrate LD and LC50

    * Microbial

- Biochemical - i.e. Immunological (ELISA); Enzyme (PPIA); Cell Receptor

- Analytical - HPLC, MS, NMR

- Genetic – PCR

  * 16S rRNA

  * nifH (gene for dinitrogen reductase-N2 fixation)

  * cpcBA, IGS (intergenic spacer region between two phycobilisome subunits),

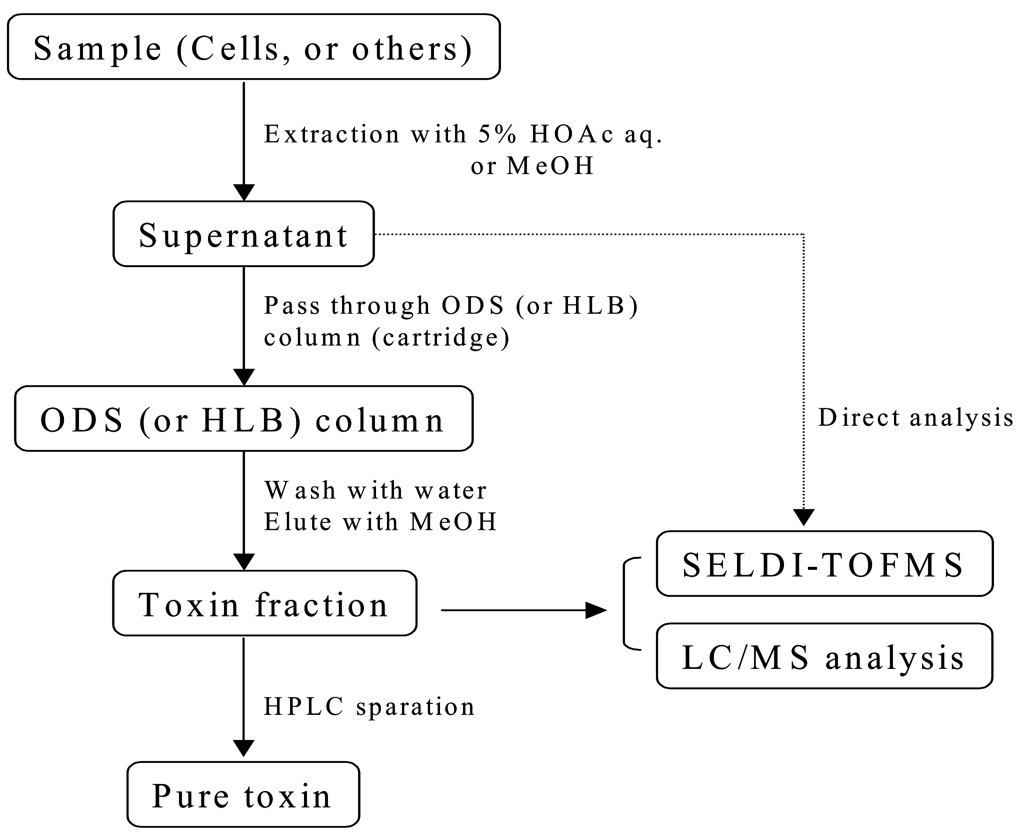
  * MCY (microcystin gene)

  * Other toxin genes (CYN, Antx-a) are being studied

Recent Methods for Analyses of Microcystins

A current method used in our laboratory for extraction and analyses of Microcystins is given in Fig. 1.

Fig. 1. Flow diagram for extraction and analyses of Microcystins.



LC/MS and MS/MS are done using an LCQ quadropole ion-trap mass spectrometer (Thermoquest, USA). The mass spectra are obtained using direct infusion or flow injection with MeOH/H_2O (1:1) mobile phase. The positive or negative ESI mode is used. For microcystins collision energy of 35-40% for MS/MS is used. Figure 2 shows the typical LC/MS conditions for microcystins.

Fig. 2. LC/MS Conditions for Analyses of Microcystins

LC conditions

Mobile phase:

ACN/0.02%HFBA H_2O/0.02%HFBA

or CAN/0.05%TFA H_2O/0.05%TFA

Column:

C18 (3μ, 050x020, MetaChem)

or SYMMERY C8 (3.5μ, 100x021, Waters)

Gradient conditions:

0-1 min: 20% organic

2-7 min: 60% organic

7.1-15 min: 20% organic

Flow rate:

0.2 ml/min.

MS conditions

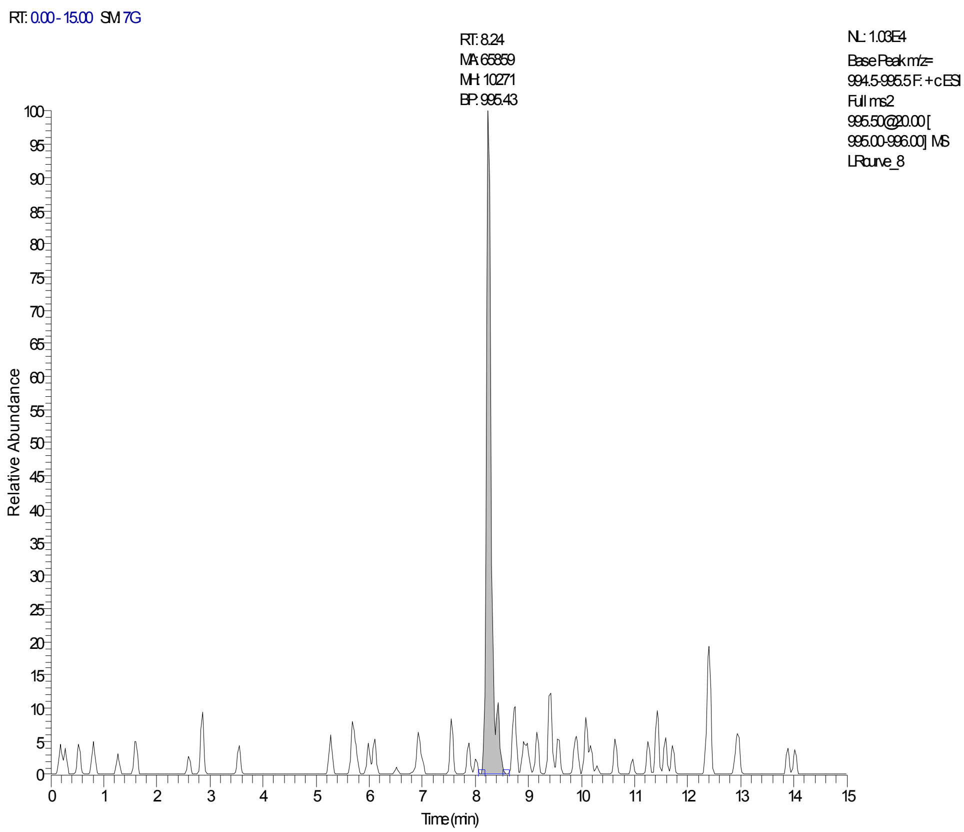
Positive or negative ESI

Source voltage: 5.0kV

Capillary temperature: 250ºC

Using these conditions microcystin-LR can be detected on column, in the positive mode, at less than 10 pg (Fig. 3).

Fig. 3. Chromatogram of 15.6 pg microcystin-LR on column (3µ C18).



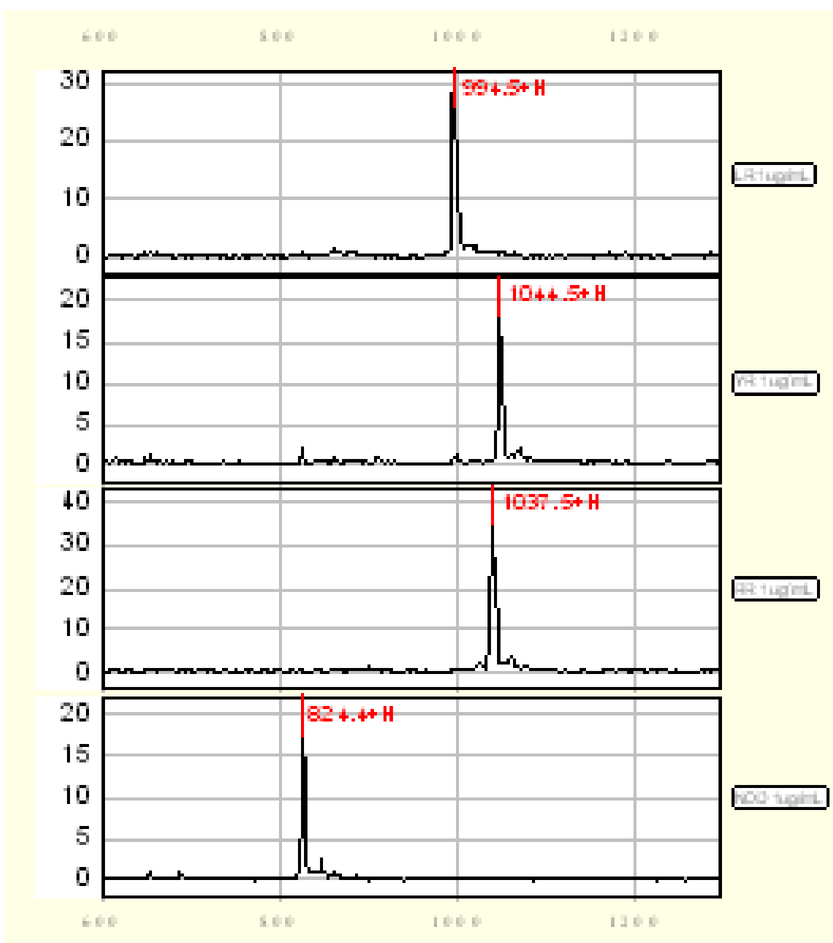
We have developed a rapid and efficient method to analyze microcystins/nodularins using surface-enhanced laser desorption ionization-time-of flight mass spectrometry (SELDI-TOFMS) with Ciphergen protein chip reader system. SELDI-TOFMS is a novel approach that was introduced by Hutchens (Hutchens 1993). This technique retains the target compounds on a solid-phase chromatographic surface (chip), subsequently ionized by laser and detected by TOFMS. The chip system with SELDI-TOF MS allows for peptide and protein analysis with high sensitive from a variety of complex biological materials such as cell, serum, blood, plasma, and tissue, et al., with limited sample preparation (Jssaq 2002).

SELDI-TOF MS analysis was carried out using the Ciphergen protein chip system (Ciphergen, Palo Alto, CA). Ciphergen Protein Chip Software 3.0 was used. The analysis procedure was adapted from the Ciphergen users guide with the following modifications.

Two μL of sample diluted in distilled water were applied to the spot on the chip and allowed to air dry. This was followed by washing with 3 μL of distilled water and allowed to dry. To the dry spot, 0.7 μL of Energy Absorbing Molecule (EAM) was added. The sample chip was transferred to the chip reader and analyzed following desorption of the bound target by short intense probes from an N2 320 nm-UV laser. The mass spectra were determined by time-of-flight in a mass spectrometer.

An example of the results for SELDI-TOF mass spectra of microcystins-LR, -YR, -RR and Nodularin are shown in Figure 4.

Figure 4. SELDI-TOF mass spectra of microcystins-LR, -YR, -RR and Nodularin. Chip: H4. Sample concentration: 1 μg/mL each. Sample volume: 2 μL. Laser intensity: 190. EAM concentration: 30% saturated CHCA solution



SELDI-TOF MS has also been used in our laboratory to analyze microcystin bound to the protein phosphatase enzyme complex. This is useful in allowing the time course of microcystin binding to be observed in vitro.

These and other methods being developed in our laboratory and others around the world will provide new techniques for observing not only microcystins but also other cyanotoxins in environmental matrices. Results obtained from these methods will help document the growing incidence of cyanotoxins in water based disease and provide the basis for water management techniques designed to improve water quality in order to minimize the human and animal health impacts from CyanoHABs.

Acknowledgements

Past and present work on CyanoHABs has involved many students, staff and colleagues. The following agencies and persons supported the work summarized in this paper:

- Florida (Funding sources)-FMRI and SJWMD through FMRI
- Wright State Univ.-Charles Friday, Jerome Servaites, Moucun Yuan, John Blakelock, Jennifer Ott, Laurel Carmichael
- UNC-IMS-Hans Paerl and crew
- Canada-E.A.D. Allen
- Univ. Illinois-Ken Rinehart-NIH Subcontract
- Australia-NRCET-Glen Shaw, Geoff Eaglesham

References

Burch, M.D., Chow, C.W.K., Hobson, P. 2001. Algicides for control of toxic cyanobacteria. Proceedings of the 2001 Water Quality Technology Conference, American Water Works Association, Nashville Tennessee.

Burch, M.D., Velzeboer, R.M.A., Chow, C.W.K., Stevens, H.C., Bee, C.M., House, J. 1998. Evaluation of copper algaecides for the control of algae and cyanobacteria. *Urban Water Research Association of Australia, Research Report, No. 130*, April 1998. UWRAA, Melbourne.

Carmichael, W.W. 1997. The Cyanotoxins. in Advances in Botanical Research (ed Callow, J.) 27, 211-256 Academic Press, London.

Carmichael, W.W. 2001. Health Effects of Toxin Producing Cyanobacteria: "The CyanoHABS". Human and Ecological Risk Assessment. 7(5): 1393-1407.

Carmichael, W.W., Azevedo, M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eagelsham, G.K. 2001. Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins. Environmental Health Perspectives. 109 (7):663-668.

Carpenter, E.J., Carmichael, W.W. 1995. Taxonomy of Cyanobacteria. in Hallegraeff, G.M. et al. (eds.) Manual on Harmful Marine Microalgae p. 373-80. IOC Manuals and Guides No. 33 UNESCO.

Casitas Municipal Water District .1987. Current Methodology for the Control of Algae in Surface Reservoirs. American Water Works Association, Denver, CO.

Chorus, I., Bartram, J. (eds.) 1999. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. World health Organization, E&FN Spon, Routledge, London.

Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (eds.) 1995. Manual on Harmful Marine Microalgae 551 pp. IOC Manuals and Guides No. 33 UNESCO.

Hutchens, T. W.; and Yip, T. T. 1993. New desorption strategies for the mass spectrometric analysis of macromolecules. Rapid. Commun. Mass Spectrom. 7: 576-580.

Jssaq, H. J.; Veenstra, T. D.; Conrads, T. P.; and Felschow, D. 2002. The SELDI-TOF MS Approach to proteomcs: protein profiling and biomarker identification.

Biochem. Biophys. Res. Commun. 292: 587-592.

Jochimsen, E.M., Carmichael, W.W., An, J.S., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., Antines, M.b. de C., Filho, D.A. de Meb, Lyra, T.M., Burreto, V.T.S., Azevedo, S.M.F.O. and Jarvis, W.R. 1998. Liver Failure and Death Following Exposure to Microcystin Toxins at a Hemodialysis Center in Brazil. New Eng. J. Medicine. March 26, 1998, V. 338(13): 873-878.

Moore, G.T., Kellerman, K.F. 1905. Copper as an Algaecide and Disinfectant in Water Supplies. Bull. Bureau Plant. Indus. U.S.D.A. 76: 19-55.

Ressom R., Soong, F.S., Fitzgerald, J., Turczynowicz, L., Saadi, O.E., Roder, D., Maynard, T., Falconer, I. (eds.) 1994. Health Effects of Toxic Cyanobacteria (blue-green algae) National Health and Medical Research Council, Australian Govt. Pub. Service, Canberra.

Sawyer, C.N. 1962. Causes, Effects and Control of Aquatic Growths. J. WPCF. 34: 279-288.

Sivonen,K. 1990. Toxic Cyanobacteria in Finnish Fresh Waters and the Baltic Sea. Ph.D. Academic Dissertation in Microbiology, University of Helsinki, Finland.

Schwimmer, D., Schwimmer, M. 1964. Algae and Medicine. in Algae and Man. D.F. Jackson (ed.) Plenum Press, New York, pp. 368-412.

Schwimmer, D., Schwimmer, M. 1967. Medical Aspects of Phycology. in Algae, Man and the Environment. D.F. Jackson (ed.) Syracuse University Press, pp. 279-358.

Yoo, R.S., Carmichael W.W., Hoehn, R.C., Hrudey, S.E. (eds.) 1995. Cyanobacterial (Blue-green Algal) Toxins: A Resource Guide 229 pp. AWWA Research Foundation and American Water Works Association, Denver.

CYANOBACTERIA TOXINS:
Florida Department of Health, Bureau of Laboratories Activities

James E. Evans, Ph.D.
Marek Pawlowicz, Ph.D.

History of cyanobacteria toxin testing at the Department of Health, Bureau of Laboratories

Bureau of Laboratories involvement in cyanobacteria testing began soon after the creation of the Florida Harmful Algal Bloom Task Force (HABTF) in 1998. In response to a HABTF Technical Advisory Group report identifying cyanobacteria as a research priority, the St. John's River Water Management District (SJRWMD) initiated a collaborative study to characterize and identify algal toxins in Florida's surface waters. Participants in this study included the SJRWMD, Wright State University, the Florida Department of Health, the Florida Marine Research Institute, and the Florida Fish and Wildlife Conservation Commission. The survey of Florida's surface waters was conducted in 1999. This survey was followed up the following year with a monitoring of surface waters identified in the 1999 survey, selected water treatment plants, and bloom events.

The Florida Department of Health, Bureau of Laboratories Environmental Microbiology Unit – Jacksonville, performed enzyme linked immunosorbent assays (ELISA) for microcystin and mouse bioassays for general toxicity in support of the initial surveys as well as the follow-up studies. The findings of these studies are undoubtedly documented elsewhere in this series of monographs and will, therefore, not be repeated. However, the cumulative findings led the Department of Health to a decision that cyanobacteria toxins were an issue requiring further investigation from a public health perspective. To adequately support future public health investigations, it was determined that laboratory capabilities needed to be expanded to allow for more comprehensive and quantitative analyses. To this end, funding for the enhancement of laboratory capacity was sought and obtained via supplemental funding from a CDC/Florida Cooperative Agreement (Pfiesteria-Related Illness Surveillance and Prevention Grant #U50/CCU415409-04).

Selection and implementation of new analytical methodology

In the selection of analytical instrumentation to enhance and expand current laboratory capabilities, the Bureau of Laboratories had a number of needs in mind. The first was to expand capabilities to include other toxins of interest beyond the microcystins. There was also a desire for more sensitive, specific, and reproducible techniques with methodologies that could identify and quantitative multiple analytes/analogs in a single analytical run. Finally, with considerations for the future, it was desired that any newly acquired capabilities conform to accepted practices and be adaptable to potential monitoring and regulatory programs.

ELISA has distinct advantages in that procedures are relatively straightforward and that assays are available in commercial kit form. In addition, ELISA methods can prove to be very sensitive, however, such claims are often not demonstrated in routine use. ELISA methods are also often plagued by analog cross reactivity. Such non-specificity may or may not be well characterized, complicating the interpretation of results. Reproducibility is also a problem with ELISA assays as they are highly dependent on technique and the extent of sample preparation required. While ELISA has proven itself as a valuable screening assay, the laboratory sought to move toward a more quantitative analytical approach.

The mouse bioassay has also proven to be a valuable screening tool for general toxicity of water containing cyanobacteria toxins. However, the assay is inherently non-specific and may be complicated by other unknown contaminants in a given water sample. It is also a non-quantitative assay, having proven to be difficult to correlate with the amount or type of toxin present. There are also concerns with cost, ethics, and relevance to human toxicity that limit the mouse bioassay's utility.

Given the limitation of the past screening assays used and in consideration of the outlined needs, the laboratory chose an analytical instrument system that provided a comprehensive solution. The system is comprised of high performance liquid chromatography with multiple detection components. Both photo diode array and fluorescence detectors as well as a mass spectrometer will be available to offer a wide range of capabilities for the detection of the various cyanobacteria toxins. Such a multiple detector set-up was designed to allow for the analysis of toxins by the currently accepted analytical methodology as well as for the development of techniques to determine multiple toxins and their analogs within the same analytical run. This system provides specific, sensitive, quantitative, and reproducible analytical capability that was lacking with the screening assays. There are limitations in that standard control material must be available to properly quantitate a given toxin, however, such standards are becoming more readily available as interest and research in the field advances. An additional limitation that bears mention, as with any quantitative technique for the cyanobacterial toxins, is that the relationship between concentration and human health effects is unknown.

Future plans

While the immediate plans for the Department of Health, Bureau of Laboratories focuses around implementing the HPLC analysis system previously described, other ongoing laboratory activities will allow for the investigation of other assay and analytical techniques. Alternate analytical techniques such as gas chromatography - mass spectrometry (GC-MS), and liquid chromatography – tandem mass spectrometry (LC-MS/MS) are available for examination of applicability to toxin analysis. In addition, bioassay and molecular analyses currently in use in other public health initiatives within the laboratory allow the possibility of applying these techniques as well.

In addition to the investigation of other assay and analytical methodologies, the addition of laboratory capabilities allows for the assumption of projects to consider the various public health implications of cyanobacteria toxin exposure. One such study supported by the CDC/Florida DOH Cooperative Agreement (Pfiesteria-Related Illness Surveillance and Prevention Grant #U50/CCU415409-04) is a study designed to determine the effectiveness of home filtration devices in the removal of cyanobacteria toxins from potable water. This study will seek to identify filter matrices that are effective in the removal of different cyanobacteria toxins. It is expected that the ability of a given filter type to effectively remove toxin will be dependent upon such variables as the type of toxin, filter composition, filter design, filter capacity, filter size, and water quality to name just a few. The results of this study could provide a basis for advising consumers how to prevent or minimize exposure to cyanobacteria toxins in the absence of standards or scientific evidence of actual health risks.

Conclusions

In conclusion, the Department of Health Laboratory is expanding capacity and capability to perform analyses for cyanobacteria toxins. The laboratory is committed to working with public health partners to address issues surrounding cyanobacteria toxin exposure and possible links to human health effects.

The laboratory wishes to acknowledge the CDC/Florida Cooperative Agreement (#U50/CCU415409-04), the Florida Harmful Algal Bloom Task Force, and the Department of Health, Division of Environmental Health, Bureau of Environmental Epidemiology for their support.

The authors also wish to acknowledge work and dedication of the staff of the Environmental Microbiology Laboratory, Environmental Chemistry Laboratory, and the entire Florida Department of Health, Bureau of Laboratories in their continued efforts to protect public health by providing diagnostic screening, monitoring, reference, emergency, and research laboratory services.

Current EPA Work on Algal Toxins in Drinking Water

James L. Sinclair
Office of Ground Water and Drinking Water
U.S. EPA
Cincinnati, OH

The Safe Drinking Water Act Amendments (SDWA) of 1996 (PL 104-182) authorizes regulation of previously unregulated drinking water contaminants in public water systems. This act requires that a list of contaminants (Contaminant Candidate List, or CCL) to be considered for regulation be published 18 months after enactment of the SDWA (1998) and every 5 years thereafter. Contaminants placed on the list are not currently regulated, are known or thought to occur in public water systems, and may require regulation. At least five contaminants are required to be considered for regulation every 5 years. Contaminants may be regulated if they have an adverse health effect on people, are known to occur or there is a substantial likelihood that they will occur in public water systems, and regulation presents a meaningful opportunity for health risk reduction in people served by public water systems.

The Federal Register notice announcing the Contaminant Candidate List (63FR10274) includes a list of research topics where sufficient information is required to make a regulatory determination on contaminants. These topics include health effects of contaminants, control of contaminants by drinking water treatment, occurrence in water, and analytical methods that are used to get occurrence information. Specific contaminants are listed as needing research in one or more of these areas before a regulatory determination can be made. For algae and their toxins, research is needed on health effects, treatment, occurrence and analytical methods.

The research that is needed to make regulatory decisions may be done through research organizations that include research facilities of the EPA. Additionally, in the case of contaminant occurrence, occurrence information may be gotten from surveys carried out by these research organizations or it may also be gotten from a public water supply monitoring regulation, the Unregulated Contaminant Monitoring Rule (64FR50555, Scharfenaker, 2001). The UCMR requires that certain public water supplies monitor for selected contaminants for one year or less, depending on the monitoring option used. The contaminants to be monitored under the UCMR are picked from the CCL. The UCMR monitoring list is revised every 5 years on a schedule where it is published one year later than the CCL. The first UCMR monitoring list was published in 1999, and the second UCMR list will be due in 2004.

Research on algae and their toxins in the required areas has been planned or initiated at several EPA research laboratories. Health effects projects being done at EPA's National Health and Environmental Effects Research Laboratory (NHEERL) in Research Triangle Park, NC, includes a project with Wright State University to study biomarkers of exposure to microcystins. The same group also recently completed a project analyze microcystins in samples from Brazilian patients who received dialysis water containing microcystins. A different laboratory of NHEERL is looking at the effects of anatoxin-a on the behavioral responses of rats. This work may be expanded to the affect of anatoxin-a on mouse behavior, and future work may be done on behavioral responses of laboratory animals to saxitoxins. The EPA NHEERL laboratory in Gulf Breeze, FL, is conducting a project with the University of Miami to look at the affect of long term exposure of fish to microcystin and cylindrospermopsin in two Florida lakes in which each of these cyanobacteria is a dominant species.

EPA projects on the affect of water treatment on algal cells or toxins has been started or proposed in three areas. The EPA's National Risk Management Research Laboratory (NRMRL) in Cincinnati is beginning a project on filtration of algal cells. This project will look at removal of cells during filter ripening, at the beginning of the filter cycle, and during breakthrough, at the end of the filter cycle. The filter will be a dual media sand and anthracite filter. Three species of cyanobacteria will be used, including *Microcystis* which is unicellular, and *Anaebena* and *Planktothrix,* which are filamentous. The experimental variables will be the run time, and the loading rate. NRMRL in Cincinnati also has a treatment project with University of Wisconsin, the Wisconsin State Laboratory of Hygiene, and Lake Superior State University. This project will look at the affect of chlorine on microcystin LR and anatoxin-a. and the influence of pH on toxin fate during exposure to chlorine. The experiments may include the affect of chlorine on intracellular and extracellular toxins. An investigator of the Office of Ground Water and Drinking Water in Washington, DC, has proposed studying the fate of algal toxins during bank filtration. The primary means of reducing the toxins would be biodegradation of toxins by microorganisms in riverbank sediments. This project has yet not been funded.

EPA has several analytical method development projects planned or under way, however, before method development can begin, analytical chemistry standards are needed. The EPA's Office of Ground Water and Drinking Water (OGWDW) Technical Support Center (TSC) in Cincinnati is funding work at Wright State University to obtain cylindrospermopsin and anatoxin-a standards. The TSC has still not located a source of microcystin LA. The National Exposure Research Laboratory (NERL) in Cincinnati is doing a project to develop a single micro LC/MS method for several microcystin congeners, anatoxin-a and is planning to work on cylindrospermopsin if a standard can be gotten. Separation will be done with microbore HPLC and electrospray time of flight mass spectrometry will be used for detection and identification. OGWDW, TSC, in Cincinnati, is starting a project to develop an LC/MS method that will be standardized and validated so that it can be run by commercial labs that analyze samples for the EPA's Unregulated Contaminant Monitoring survey for algal toxins. A concentration and clean-up step will be needed before analysis.   The goal will be to make a single LC/MS method for 5 or 6 microcystins, anatoxin-a and cylindrospermopsin. OGWDW, TSC, in Cincinnati also is funding the initial steps toward development of immunoassay kits for cylindrospermopsin and anatoxin-a through Wright State University. Conjugates and haptens will be developed and given to a private company for development of the immunoassay kits. The National Center for Environmental Research (NCER) in Washington, DC, also offered a small business grant under their SBIR program for the development of these kits in 2002. NERL in Cincinnati is working with a company, Optec, to make an automated instrument for detection of algal toxins which uses aptamers. Aptamers are oligonucleotides that bind to specific molecules including algal toxins. They can be regenerated and reused. NERL in Cincinnati is planning to develop a taqman PCR method for identification of 6 different genera of toxic cyanobacteria. This method would give results in 2 to 3 hours and would be quantitative. OGWDW, TSC in Cincinnati is developing real-time PCR for a microcystin gene. This method will be used in a project to look at microcystin production under differing environmental conditions. OGWDW, TSC in Cincinnati is starting a project with Lake Superior State University. has been working on a standardized microscopic cell count method that could be used for all of the toxic genera of concern. This method will be used with the UCMR survey.

EPA would like to conduct a risk assessment for algal toxins, if resources are available. The National Center for Environmental Assessment (NCEA) in Cincinnati would like to look at quantitative structure-activity relations for cylindrospermopsin and microcystins. NCEA is also interested in doing a risk assessment for the microcystins, cylindrospermopsin and anatoxin-a for the United States.

EPA has done or is doing 3 small occurrence surveys for algal toxins in the last 2 years. NERL in Cincinnati has worked with USGS to survey 2 lakes and a river during the summer of 2001 for microcystin. The samples were archived and haven't been analyzed yet. OGWDW, TSC in Cincinnati with Lake Superior State University did a survey in the summer of 2001 for microcystin. Cell counts were also done. The samples were archived and will be analyzed this summer. This summer OGWDW, TSC is conducting a survey with Lake Superior State University for cylindrospermopsin. Source water and treated water are being surveyed at 6 utilities. These were picked based on their past history of having cylindrospermopsin in the water. The samples from this survey are being archived until there is an analytical capability for cylindrospermopsin.

EPA has a separate regulation to get occurrence information on CCL contaminants in drinking water systems. This is the Unregulated Contaminant Monitoring Rule (UCMR). Algal toxins weren't monitored under the first UCMR which goes from 1999 to 2004 because there were no validated methods that could be used for the survey. It is likely that they will be monitored under the next UCMR monitoring list, or UCMR2, which is due to be promulgated in 2004. Since it takes at least a year to write regulations that are published in the Federal Register, to meet the 2004 deadline for publishing UCMR2, it will be necessary to have validated methods for the UCMR by the end of 2003 or early 2004. Monitoring will probably be done for the toxins that have standards available. This includes 5 or 6 microcystins, cylindrospermopsin, and anatoxin-a. Based on the status of analytical methods, it is likely that there will be an LC/MS method and probably a microscopic cell count method in time for UCMR2 in 2004, but screening methods for the toxins, and genetic methods for toxin gene identification won't be ready in time. Based on the cost of the analyses, the UMCR survey for algal toxins will probably be small, including 300 or fewer systems.

Therefore, these projects show that EPA is implementing research to get the information it needs to make a regulatory determination for algal toxins although it still doesn't have all the needed information.

The compilation of the second CCL will be very important for future studies of algal toxins, because algal toxins could be kept on the CCL or they could be dropped off of it which would affect EPA research and regulatory activities. The second CCL is due to be published in February 2003, and it will likely be very similar to CCL1. Future CCLs could be different, though. For future CCLs, EPA must consider an approach recommended by the National Research Council for picking CCL contaminants. The strategy that the NRC recommended was to start with a universe of about 80,000 contaminants and pare the list down to a pre-CCL by using a neural network program in conjunction with expert judgment. The NRC also recommended screening potential microbial contaminants for the pre-CCL by characterizing them with quantitative structure activity relations for chemicals, or virulence factor activity relations or VFARs for microorganisms. The VFAR approach is to detect virulence factors that microorganisms have. These virulence factors could be detected either by genotypic or phenotypic expressions. This approach could affect the selection of algal toxins for future CCLs since the selection criteria could be different than those used to pick CCL1 contaminants. For CCL2, though, it is likely, though not assured, that algal toxins will remain on the CCL, and will continued to be considered for a regulatory determination.

Reference

Scharfenaker, M.A. 2001. Making sense of the Unregulated Contaminant Monitoring Rule. JAWWA 93(6):24-42

Summary of Florida Department of Environmental Protection's Activities Related to Cyanobacterial Toxins

Presented by: Richard Drew, M.S. Chief, Bureau of Water Facilities Regulation, Division of Water Resource Management

The Department of Environmental Protection [DEP] has several programs that either influence the presence or reoccurrence of cyanobacteria and their associated toxins or are impacted by the presence of the cyanobacteria. I will limit this discussion to two programs that influence the nutrient balance of Florida surface waters that in turn create a suitable environment for that group of organisms; and one program, drinking water, that is directly affected by its presence.

The NPDES or National Pollutant Discharge Elimination program regulates the treatment and disposal of industrial and domestic wastewater. Specific effluent limits control the amounts of nutrients that can be discharged to surface waters, that in turn could promote eutrophication and the presence of cyanobacteria. The nutrient limits contained in facility permits are based on compliance with water quality standards, and the standards are related to the designated use or classification of the water body receiving the discharge.

Direct wastewater facility discharges to shellfish areas [Class II] and to water bodies that serve as a source of drinking water [Class I] are prohibited in Florida. Even indirect discharges must demonstrate that what will be discharged will not affect the designated use of the downstream waters. While there are prohibitions for direct discharges to Class I and II waters, recreational waters [Class III] can receive discharges from wastewater facilities. However, these discharges, like indirect discharges to Class I and II waters, are only allowed if it is demonstrated by the facility permit applicant that they will not affect the designated use of the water body. In relation to cyanobacteria, the permit applicant must demonstrate that the nutrients added to the receiving water will not cause algae-related pigments to exceed levels found in healthy waters. Therefore, the NPDES program has the ability to limit nutrient additions for any wastewater facility in the state.

The remaining nutrient sources reaching surface waters and promoting algae blooms are derived from non-point sources, such as storm water runoff from construction areas, urban storm water, poorly operated residential wastewater treatment systems [septic tank systems], irrigation from agriculture/right of ways/golf courses, and atmospheric deposition. The TMDL or Total Maximum Daily Load program examines all water bodies in the state and for those determined to be impaired a process is set in motion. First the appropriate levels of pollutants are defined that will protect the designated use of the water body; then the sources of those pollutants are identified; and finally, an implementation plan is developed to reduce the loading to the appropriate level. It is in this program that the agency hopes to control all the nutrient sources supporting the occurrence of algae blooms. Here the concept of watershed management is applied to reduce nutrient loadings from all the sources in the basin.

A DEP program that can be directly affected by the presence of cyanobacteria and their toxins is the drinking water program. Fortunately the potential for this to become a problem in Florida is greatly reduced by a vast system of aquifers that provide ground water to a majority of Florida's population. Only 20 of about 6,400 public water systems (PWSs) use surface water. These 20 PWSs supply roughly 10% of Florida's drinking water. Geographically, only one of the 20 systems is located in the northern part of the state. The rest are south of Orlando. Most are located within a 50-mile wide band running from West Palm Beach on the eastern coast to Charlotte Harbor and Ft Myers to the west. Most of these surface waters are slow moving and affected by water control structures. Fluctuating levels of organic color, iron, and acidity affect the quality [taste and odor] of the water and dictate the level of treatment used at these sites.

Drinking water systems [WTPs] are required to meet a number of federal regulations that have been adopted by Florida. The regulations are even more complex for systems that draw their source water from surface waters. The treatment processes used at surface WTPs is based on the quality [including taste, clarity, and odor] of the water supply. As a result these systems are typically very responsive to changes in the source water character and take steps quickly to minimize citizen complaints. In regard to public notification, the Department requires annually the production and public distribution of Consumer Confidence Reports that summarize the quality of the water provided to the public. Recently, the EPA and in turn the DEP have adopted more stringent rules focused specifically on systems that use surface waters as their source water. Surface Water Treatment, Filter Backwash Recycling, and Stage 1 Disinfectants/Disinfection Byproducts Rules are examples of these regulations.

While there are no regulations that directly address the treatment of cyanobacteria and its toxins or establish specific water quality standards for these toxins, the water treatment plant must satisfy color and odor requirements of DEP and EPA. To this end, the facility must adopt a set of management strategies to handle the changing water quality of the surface water body serving as its source. Many of these management strategies have also been shown to be effective at removing or controlling cyanobacteria and its t toxins.

Management strategies are site specific and can be categorized as controlling the impacts to the source water, pretreatment practices, and in-plant and post-plant treatment techniques.

Watershed management was previously discussed and attacks the source of the problem – nutrients. However, depending on the size of the watershed feeding the source water, the implementation of watershed management techniques may be: impractical [technically or financially] in controlling nutrient loads to the basin; or, impractical in achieving long term results; or, may not be as effective as originally hoped even over the long term. This leaves the WTP with the entire responsibility of handling the algae bloom problem.

Pretreatment practices include physical, chemical or biological treatment of the source water *in situ,* or prior to entering the facility's treatment process train. Some in-lake management techniques, such as herbicide applications, are merely temporary fixes, attacking the problem of algae blooms as they happen. Use of a herbicide or algalcides [e.g., copper sulfate] can be most effective when used to maintain low numbers of algae, instead of application on a bloom when it can release toxins from the dead cells. It also appears to be less effective with continued use in the same water body.

Other types of lake management practices either try to remove the nutrients from the water [chemical coagulants] or create an unsuitable environment for the development of a bloom, [aeration, pH adjustment]. Another technique is the use of off-line wetland treatment systems that filter [biologically or physically] nutrients out of the water and into wetland vegetation and sediments.

A drinking water treatment facility can also develop alternative sources of water to be used when blooms appear in their primary drinking water supply, i.e., above ground reservoirs, aquifer storage and recovery systems, backup wells, use of adjacent water utility water.

Treatment at the plant, particularly those facilities drawing surface waters, can involve several filtration treatment techniques that will effectively remove the algal cells, including in-bank filtration, coagulation and filtration, flocculation and filtration, and other forms of membrane filtration, such as reverse osmosis and nanofiltration. Some WTP practices applied during blooms, such as the isolation of sludge and filter backwash water to waste can prevent the reintroduction of cells and toxins into the treatment process train. Disinfectants commonly used for pathogen removal, like chlorine and ozone can be partially effective at removal of the certain toxins. Powdered and Granular Activated Carbon [PAC & GAC] can be effective measures at removing some of the toxins, particularly PACs. Combining PAC or GAC with one of the disinfectants can improve the removal efficiency. Some oxidants, like potassium permanganate, have also been shown to be especially effective at removing the toxins.

Surface waters in Florida commonly used as source waters are, unfortunately, often high in color and other organics, and have odors characteristic of stagnant wetlands. Fortunately, the techniques used to produce a good tasting, odor-free, clear drinking water, and compliant with DEP/EPA drinking water quality standards and new regulations, require employment of a series of treatment techniques that also seem to be very effective at removing cyanobacteria and theirs associated toxins.

The evaluation of treatment techniques to effectively remove cyanobacteria and their toxins is in the early stages of discovery, as evidenced in recent publications [Chorus and Bartram[Ed.], 1999; Carmichael, 2001; Chorus[Ed.], 2001]. Ways to measure the presence of toxins, especially at the low concentrations observed in the environment are still being developed. Standardization of analytical methodologies and sampling protocols must be done to allow the WTP to have tools for detecting the toxins and have assurance that the values reported, for the increasing number of toxins, are accurate.

Several systems in Florida are participating in current studies funded by the American Water Works Association Research Foundation and the St. Johns River Water Management District to examine the occurrence, quantity, and treatability of algal toxins at drinking water treatment systems. In addition, DEP is working with the surface water WTPs in a workgroup format to enhance the exchange of experiences each of the WTPs is having in dealing with this and other related problems. EPA is also evaluating cyanobacteria-derived group of toxins to determine if they will establish a maximum concentration level [MCL] for one or more of the more common cyanobacters.

Where does this leave DEP, as regulators? On one hand, the information to date for Florida WTPs leads one to conclude only a small portion of the state's drinking water systems are susceptible to potential introduction of cyanobacteria and its toxins. A high level of treatment is now being provided at these few systems caused by the nature of the water they now treat, and this treatment based on the research to date appears to be effective at controlling the introduction of toxins into the distribution systems. On the other hand, a fuller understanding of the problem and the effectiveness of treatment is still evolving. We should monitor this information and disseminate the results to the WTPs as soon as possible. We should also continue to work with the TMDL program to control the nutrient inputs to the watersheds containing the surface waters supplying these WTPs. Controlling and lowering the nutrients will reduce and hopefully eliminate the development of these algae blooms and the release of their toxins.

References:

Carmichael, W.W., 2001. ASSESSMENT OF BLUE-GREEN ALGAL TOXINS IN RAW AND FINISHED DRINKING WATER. AWWARF Public. No. 90815. 179pgs.

Chorus, I., and J. Bartram, eds. 1999. *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management.* London, UK: E&FN Spon. 416pgs.

Chorus, I., ed. 2001. *Cyanotoxins: Occurrence, Causes, Consequences.* Berlin: Springer. 357pgs.

Aquatic Toxins Program
Florida Department of Health
Richard Clark, MS

On August 13-14, 2003, the Florida Department of Health hosted a meeting in Sarasota, FL, entitled *Health Effects of Exposure to Cyanobacteria Toxins: State of the Science*. Many cyanobacteria researchers, water treatment plant personnel, and health care professionals gathered to exchange information and further Florida's knowledge on this new area of science. The Florida Department of Health established their Aquatic Toxins Program in 1997 to track illnesses from persons exposed to algal toxins. The program created the Aquatic Toxin or Harmful Algal Bloom Hotline (888-232-8635) at that time and began tracking illnesses and exposures to harmful algae. This program also participates in many research and educational activities.

Aquatic Toxins Surveillance

The surveillance activities of the program began in response to *pfiesteria* concerns in 1997. *Pfiesteria piscicida* is an estuarine dinoflagellate that is believed to cause health problems in humans, but the results of years of research are conflicting and it has not been determine if *pfiesteria* actually produces a toxin. Florida does have *pfiesteria* in its estuarine water bodies, but we have had no confirmed illnesses related to exposure to this organism.

The Aquatic Toxins Program also works closely with the Food and Waterborne Illness Program to track illnesses of people who have been exposed to an algal toxin through food sources. Florida has cases of Neurotoxic Shellfish Poisoning (NSP), ciguatera fish poisoning, puffer-fish poisoning and scombroid fish poisoning each year. Each of these illnesses is due to a toxin that is of algal or bacterial origin.

Persons can also become ill from direct contact with the algal toxins. Skin rash, nasal and throat irritation, headache, and breathing problems can be associated with Red Tide (*Karenia brevis*) and cyanobacteria. The program receives hundreds of reports of illnesses from persons exposed to red tide each year. So far, there have been no confirmed reports of illness related to cyanobacteria in Florida.

Research Activities

The Aquatic Toxins Program is an active participant in many research projects in Florida. We collaborate on Red Tide studies with The National Center of Environmental Health, Centers of Disease Control and Prevention, University of Miami Medical School, Lovelace Respiratory Institute, Florida Department of Environmental Protection, University of North Carolina Wilmington, and Mote Marine Lab. The goal of these human health studies is to establish how red tide affects the environment and people. The studies involve environmental monitoring, air monitoring, and human health monitoring including: nasal and throat swabs, urine test, spirometry and questionnaires.

The Aquatic Toxins Program was also able to support the Department of Health's Laboratory in Jacksonville, FL, in building capacity to test water for cyanobacteria and their toxins. The equipment is now in place and their first research project is underway. This project is testing how effective home filtration devices are at removing cyanobacteria and their toxins.

The Aquatic Toxins Program will also be participating in a puffer-fish study with The Florida Marine Research Institute. Nineteen people had become ill after eating puffer-fish caught in the Titusville, FL, area in 2002. The fish had bioaccumulated saxitoxin in their tissue, which had not been previously identified in the literature. Research will determine the toxicity of the 16 different species of puffer-fish and how they are distributed in Florida. This project will also determine from what species of algae was the toxin's origin and what other organisms may contain the toxin.

Education

One of the main goals of the Aquatic Toxins Program is education and it has created materials for use by the county health departments, the public and the media. A "Cyanobacteria Update" is released to the county health departments when there is any new information of interest. The program is very active in providing assistance to the county health departments when they need to produce a press release related to puffer-fish or red tide.

Promotional items have been created for the public to get the hotline number out. Cyanobacteria magnets and key chains have been created along with red tide visors and key totes. Scientists and county health department staff distribute these items where people are recreating near water bodies. These items have the hotline number printed on them and they encourage people to call in if they feel they have been exposed to something in the water that has made them ill.

The coordinator of the program has given many educational presentations at national and local scientific meetings, public meetings, county health departments and wildlife parks.

Miscellaneous Aquatic Toxins Program Activities:

- Letters written from FDOH to EPA regarding setting recreational and drinking water guidelines for cyanobacteria toxins.

- Investigations into reported cases.

- Creation of the physician workgroup

- FDOH is an active member of the Florida Harmful Bloom Task Force

- Cyanobacteria Literature Collection and review

- Red Tide Literature Collection and review
- Participation in the production of professional posters and presentations to be given at scientific meetings
- Coordination of conference calls
- Response to media calls, emails, and questions from the public
- Response to reports of swimmer's itch, cercarial dermatitis, naegleria (amoeba that causes meningitis), jellyfish stings, fire coral, fish bites, etc.
- Production of DOH education plans, business plans, media contact reports, etc.
- Coordination of the State of the Science Cyanobacteria meeting: choosing speakers, setting the agenda, providing nametags, travel reimbursement, travel coordination, notebooks, and contracts with Mote and supporting agencies.

Conclusion

The Florida Department of Health's mission is to promote and protect the health and safety of all people in Florida through the delivery of quality public health services and the promotion of health care standards. The Aquatic Toxins Program follows this mission throughout its activities in education, research and health surveillance. In Florida, harmful algal blooms like red tide and cyanobacteria are common, therefore, there is a potential for human illness. The program will continue surveillance for health effects of harmful algael blooms and to support Florida's research regarding related emerging health concerns.

Summary of: Water Treatment
Presented by: Bruce MacLeod, M.S.

Mr. Bruce W. MacLeod, from the Manatee County Utility Operations Department, discussed the approach utilities take regarding cyanobacterial toxins. Mr. MacLeod began by discussing local newspaper articles related to cyanobacterial toxins. These articles, in Mr. MacLeod's opinion, are likely to frighten the public. The Manatee County facility is a large one, which often uses unique treatment schemes to address site-specific problems. General management strategies for the utilities include the prevention of blooms and the removal of toxins. Blooms can be prevented using lake aeration, algaecides, inactivation of phosphorous, lake shading, disruption and watershed management. Toxins can be removed from water by treatment with chlorine, ozone, potassium permanganate, UV and membranes. Each of these removal methods has its own problems, such as taste changes, time dependence and added costs.

The Lake Manatee facility has a number of unique characteristics that impact treatment. It has a 125 square mile watershed and an instream reservoir dating back to 1967. The reservoir covers 1800 acres and has an average depth of 12.8 feet and a volume of 12 billion gallons when full. The broad, shallow shoreline allows for algal growth on the periphery of the lake. The water is soft, acidic, highly colored and nitrogen-limited, with a total organic carbon (TOC) range from 10-30 mg/L. The temperature ranges from 15-30 °C, with temperatures above 25 °C 65% of the time. *Anabaena* and *Oscillatoria* have been found recently. Also, a Microcystis bloom was observed several years ago. Blooms are more common in the winter, which is the dry season.

Blooms have been prevented at Lake Manatee by spraying copper sulfate from an airboat, at temperatures between 21-30 °C. Problem areas are sprayed at filament counts of 2 filaments/mL, and other areas are sprayed at levels above 2 filaments/mL. Mr. McLeod's experiences have suggested that copper sulfate has had little if any impact on algal densities, with fragmentation of filaments being the only change. *Anabaena circinalis* was viable, even at copper levels of 1 mg/L. Copper may stimulate the growth of actinomycetes, and other organisms that produce taste and odor compounds.

According to Mr. MacLeod, the watershed is being managed in a number of ways, which should reduce nutrient loading. First, efforts are made to encourage best management practices (BMPs) for agricultural land uses. Homeowners on the watershed are educated about the use of xeriscaping and other low fertilizer methodologies. A watershed overlay district was established to limit housing density and prevent the use of reclaimed water. Manatee County has been purchasing watershed land since 1987, following a successful local ballot initiative. Thus far 43%, or 35,000 acres, of the watershed is publicly owned.

A major focus at Lake Manatee is odorant removal. Prior to 1988, a qualitative method to determine the proper amount of active carbon was used. This method wasted money, due to chasing the effective carbon dose and still yielded complaints from water consumers. Between 1988 and 1989, an in-house quantitative method was developed, a treatment goal was set, optimization studies were performed and odorant levels were monitored in raw and treated water. As a result of these efforts, the number of complaints decreased.

Current activities include HPLC/MS method development for anatoxin, LPS and cylindrospermopsin, bench-scale membrane studies with microcystin-spiked filter effluent at the University of South Florida, expanded toxin monitoring and powdered activated carbon (PAC) treatment studies. Membrane filters have been found to reduce microcystin-LR in water samples by over 94%, as well as 60-96% of TOC, at the bench-scale. Pilot-scale studies will be conducted at Lake Manatee by the end of 2002.

Mr. MacLeod closed by discussing water industry needs related to cyanobacterial toxins. These include acute and chronic acceptable concentrations; dependable standards for anatoxin, cylindrospermopsin and endotoxins; rapid, easy and rugged testing methods and site-specific treatment studies.