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## Cyanobacterial Harmful Algal Blooms: Chapter 2: A Synopsis of Research Needs Identified at the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB)

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## **Chapter 2: A Synopsis of Research Needs Identified at the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB)**

H Kenneth Hudnell and Quay Dortch

### **Abstract**

Evidence indicates that the incidence of cyanobacterial harmful algal blooms (CHABs) is increasing in spatial extent and temporal frequency worldwide. Cyanobacterial blooms produce highly potent toxins and huge, noxious biomasses in surface waters used for recreation, commerce, and as drinking water sources. The Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB) characterized the state of the science and identified research needed to address the risks posed by CHABs to human health and ecosystem sustainability. This chapter provides a synopsis of CHAB research needs that were identified by workgroups that addressed charges in major topic areas. The research and infrastructure needed are listed under nine categories: 1) Analytical Methods; 2) CHAB Occurrence; 3) CHAB Causes; 4) Human Health; 5) Ecosystem Sustainability; 6) CHAB Prevention; 7) CHAB Control and Mitigation; 8) Risk Assessment and; 9) Infrastructure. A number of important issues must be addressed to successfully confront the health, ecologic, and economic challenges presented by CHABs. Near-term research goals include the development of field-ready tests to identify and quantify cells and toxins, the production of certified reference standards and bulk toxins, formal assessments of CHAB incidence, improved understanding of toxin effects, therapeutic interventions, ecologically benign means to prevent and control CHABs, supplemental drinking water treatment techniques, and the development of risk assessment and management strategies. Long-term goals include the assimilation of CHAB databases into emerging U.S. and international observing systems, the development of quantitative mod-

els to predict CHAB occurrence, effects, and management outcomes, and economic analyses of CAHB costs and management benefits. Accomplishing further infrastructure development and freshwater HAB research is discussed in relationship to the Harmful Algal Blooms and Hypoxia Research and Control Act and existing HAB research programs. A sound scientific basis, the integration of CHAB infrastructure with that of the marine HAB community, and a systems approach to risk assessment and management will minimize the impact of this growing challenge to society.

## Introduction

The Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB) characterized the state of the science and identified research needed to address the risks posed by cyanobacterial harmful algal blooms (CHABs) to human health and ecosystem sustainability. The state of the science was described by invited experts who addressed specific charges for CHAB subtopics in platform sessions and authored 23 chapters of this monograph. The research needed to develop a systems approach toward the assessment and management of CHAB risks (Hudnell *et al.* this volume) were identified in seven workgroups whose members addressed specific charges and summarized their findings in additional chapters of this monograph. The workgroups were organized to address the major topic areas of: 1) Analytical Methods; 2) CHAB occurrence; 3) CHAB causes, prevention, and mitigation; 4) Cyanotoxin characteristics; 5) Human health effects; 6) Ecosystem effects and; 7) Risk assessment. The Organizing Committee realized that there was overlap between some of the topic areas, largely due to the interconnections between components on the CHAB pathway (Hudnell *et al.* this volume). Similarities among some of the charges given to different workgroups were intended to promote characterization of the interconnections from a broader diversity of perspectives. Table 1 presents the research and infrastructure needs identified by the workgroups. Research in each of the Priority Areas is briefly discussed below. More detailed discussions of the state of the science and research needs are presented in the speaker and workgroup report chapters.

Research in the nine Priority Areas identified in Table 1 was considered to be high priority over the long term. The workgroup reports designate each research need as a near-term or long-term goal. The near-term goals are those that do not require other research to be accomplished prior to addressing those goals, whereas the long-term goals are dependent upon the

completion of near-term goals or require an extended time period to complete. The need for certified analytical methods and readily available reference standards was generally acknowledged by the workgroups to be of highest priority because many other goals are dependent of the availability of methods and materials. Methods and materials were similarly given the highest priority in the HABS report, Harmful Algal Research and Response: A National Environmental Science Strategy 2005-2015, (HARNESS 2005).

The U.S. Congress reauthorized and expanded the Harmful Algal Blooms and Hypoxia Research and Control Act (HABHRCA 2004). Whereas HABHRCA originally targeted harmful algal blooms in the oceans, estuaries and the Great Lakes, the reauthorized Act mandated a Scientific Assessment of Freshwater Harmful Algal Blooms, which will: 1) examine the causes, consequences, and economic costs of freshwater HABS throughout the U.S.; 2) establish priorities and guidelines for a research program on freshwater HABS; and 3) improve coordination among Federal agencies with respect to research on HABS in freshwater environments. The research topics discussed below are intended to help identify issues that should be addressed in order to fully meet the mandates of HABHRCA.

## **Analytical Methods**

Standardized and certified methods for collecting field samples are needed to ensure that the samples consistently represent the existing environmental conditions, and that results can be compared across time and between collectors. The samples generally consist of water, plankton, invertebrates, vertebrates, or sediments. Standardized methods also are needed for sample processing, including filtration, stabilization, transportation, and storage, as well as for the extraction of cyanotoxins from complex matrices such as biological tissues and sediments.

A tiered approach toward screening environmental samples for cyanobacteria and cyanotoxins is needed to accommodate a variety of settings and purposes, and to make efficient use of resources. Initial screening methods should be designed for field settings such as water utilities or recreational water management facilities. The identification and quantification of organisms traditionally has been accomplished through microscopy, a time consuming method that requires a high level of training. Genetically-based methods should be further developed for the identification of cyanobacteria to the species level, and for the detection of genetic se-

quences involved in toxin production. Automated cell counting methods are needed for quantification. Although standard methods exist for analyzing some cyanotoxins (Meriluoto and Codd 2005), improved methods are needed for rapid, inexpensive, and reliable analyses in field settings. Enzyme linked immunosorbent assays (ELISA) or other emerging methods are needed to measure cyanotoxin levels. The methods should be sensitive to a wide variety of cyanotoxin analogues or congeners. A combination of toxin level measurements and bioassays for toxicity will indicate the total potential for toxicity from environmental exposures. A long-term goal for field analyses is to produce real time, *in situ* monitors coupled with data transmission systems. Remote sensing systems will provide early indicators of environmental conditions that favor the emergence of CHABs, as well as information on the initiation, development, and senescence of CHABs. Finally, specialized laboratories are needed to verify field results, validate results from developing techniques, and identify novel toxins. These laboratories, which may require sophisticated and expensive equipment (for example liquid chromatographs/mass spectrometers) and high levels of technical expertise, should be shared-use facilities due to budget constraints. These facilities should be capable of operating on an emergency basis to provide a rapid response to situations endangering public health.

**Table 1.** Synopsis of Research Needs for Cyanobacterial Harmful Algal Blooms

Priority Area	Needs	Results - Improved Understanding, Methods, Products & Prediction
Analytical Methods	<ul style="list-style-type: none"> <li>Standardized Methods</li> <li>Tiered Screening</li> <li>Field Methods</li> </ul>	<ul style="list-style-type: none"> <li>Sample Collection, Filtration, Stabilization, Transport, Storage Toxin Extraction from Complex Matrices</li> <li>Strategies Adaptable to Location &amp; Purpose</li> <li>Probes for Organism Identification &amp; Toxin Production</li> <li>Multiple Analogue Sensitive Toxin Identification &amp; Quantification</li> </ul>
CHAB Occurrence	<ul style="list-style-type: none"> <li>Laboratory Methods</li> <li>Consensus Taxonomy</li> <li>Nationwide Survey</li> <li>Long-term Monitoring</li> <li>Toxin Transport &amp; Fate</li> <li>Predictive Models</li> </ul>	<ul style="list-style-type: none"> <li>Improved &amp; New Techniques for Known &amp; Novel Toxins</li> <li>Consistent Taxonomic Identification to Species Level</li> <li>CHAB &amp; Toxin Occurrence in Source Water using UCMR</li> <li>CHAB &amp; Toxin Occurrence in Recreational Water</li> <li>CHAB Occurrence Trends in Source &amp; Recreational Waters</li> <li>Remote Sensing Methods &amp; Coupling with Global Observing Systems</li> <li>Environmental Transport, Accumulation &amp; Degradation</li> <li>Local CHAB Occurrences</li> <li>Toxin Production, Environmental Transport, Accumulation &amp; Fate</li> </ul>

CHAB Causes	<ul style="list-style-type: none"> <li>• Retrospective Data Analyses</li> <li>• Controlled Studies - Lab, Field, Mesocosom &amp; Mesocosom</li> <li>• Ecosystem Monitoring</li> <li>• Predictive Models</li> </ul>	<ul style="list-style-type: none"> <li>• Physical, Chemical &amp; Biological Variations Over Space &amp; Time</li> <li>• CHAB Responses to Controlled Environmental Variables</li> <li>• Identification of Toxin Production Triggers</li> <li>• CHAB Dynamics &amp; Environmental Interactions</li> <li>• CHAB Expansion with Climate Change &amp; Other Stressors</li> <li>• Driver Thresholds that Destabilize Ecosystems &amp; Induce CHABs</li> <li>• Factors Controlling CHAB Initiation, Dynamics &amp; Toxin Production</li> </ul>
Human Health	<ul style="list-style-type: none"> <li>• Human Health Effects</li> <li>• Predictive Models</li> </ul>	<ul style="list-style-type: none"> <li>• Bioindicators of Human Exposure &amp; Effect</li> <li>• Toxicokinetics, Toxicodynamics, Dose-Response Relationships</li> <li>• Epidemiology, Repeated Recreation &amp; Drinking Water Exposures</li> <li>• Routes &amp; Quantities of Human Exposure to Toxins</li> <li>• Quantitative Structure-Activity Relationships</li> </ul>
Ecosystem Sustainability	<ul style="list-style-type: none"> <li>• Ecosystem Effects</li> </ul>	<ul style="list-style-type: none"> <li>• Toxin &amp; Concurrent Stressor Effects on Key Biota &amp; Communities</li> <li>• Bioaccumulation, Bioconcentration &amp; Biomagnification in Food Web</li> </ul>
CHAB Prevention	<ul style="list-style-type: none"> <li>• Predictive Models</li> <li>• Nutrient Source Identification</li> <li>• Watershed Management</li> <li>• Water Management</li> <li>• Predictive Models</li> </ul>	<ul style="list-style-type: none"> <li>• Eutrophication, CHAB &amp; Turbidity Relationships</li> <li>• Eutrophication &amp; CHAB Driven Ecosystem Alterations &amp; Fate</li> <li>• External Inputs Versus Internal Nutrient Recycling</li> <li>• Methods to Reduce External Nutrient Input</li> <li>• Methods to Increase Flow, Destratify &amp; Increase Competitive Forces</li> <li>• Relative Effectiveness of Prevention Strategies</li> </ul>

<p>CHAB Control &amp; Mitigation</p>	<ul style="list-style-type: none"> <li>• Bloom &amp; Toxin Destruction</li> <li>• Drinking Water Treatment</li> </ul>	<ul style="list-style-type: none"> <li>• Environmentally Benign Methods to End CHABs &amp; Degrade Toxins</li> <li>• Detect Presence of Cell Fragments &amp; Toxins During Water Processing</li> </ul>
<p>Risk Assessment</p>	<ul style="list-style-type: none"> <li>• Predictive Models</li> <li>• Integrated Human Health &amp; Ecosystem Risk Assessment</li> <li>• Reduce Data Uncertainties</li> </ul>	<ul style="list-style-type: none"> <li>• Methods to Remove Fragments, Toxins, Taste &amp; Odor Compounds</li> <li>• Relative Effectiveness of Control &amp; Mitigation Strategies</li> <li>• Interdependence of Human Health &amp; Ecosystem Sustainability</li> <li>• Assessing Risks from CHAB Biomass Risk from Single Toxins</li> <li>• Cyanotoxin, Analogue Toxicity Equivalence Factors &amp; QSAR</li> <li>• Total Risk from Common Toxin Mixtures &amp; Crude Cell Extracts</li> <li>• Susceptibility Factors</li> <li>• Safe Production, Storage &amp; Transportation</li> <li>• Airborne Dispersal &amp; Route of Exposure Relative Toxicity</li> <li>• Local &amp; National Total Cost of CHABs to Society</li> <li>• CHAB Cost Versus Prevention, Control &amp; Mitigation Cost/Benefit</li> <li>• Pre- Versus -Post Prevention, Control &amp; Mitigation Cost/Benefit</li> </ul>

Infrastructure	<ul style="list-style-type: none"> <li>• Shared Centralized Facility &amp; Service</li> </ul>	<ul style="list-style-type: none"> <li>• High Complexity Equipment, Analyses, Training &amp; Certification Produce &amp; Provide Certified Toxin Standards &amp; Bulk Toxins U.S. Surveillance, Databases, &amp; Coupling with International Systems</li> </ul>
	<ul style="list-style-type: none"> <li>• Coordination</li> </ul>	<ul style="list-style-type: none"> <li>• Improved Federal, Stakeholder &amp; International Coordination Standing Fresh to Marine Advisory Committee &amp; International Link</li> </ul>
	<ul style="list-style-type: none"> <li>• Education</li> </ul>	<ul style="list-style-type: none"> <li>• Train Volunteer, Industry, Utility, Government, Academic, Post Doc Public &amp; Stakeholder Education Services</li> </ul>

## CHAB Occurrence

Although CHABs have been reported worldwide and in most or all states in the U.S., there is no international or national database that contains records of all CHAB events. The degree to which states or local governments record CHABs is highly variable. Therefore, no definitive information is available on the incidence of CHABs over time and space in the U.S., the genera and species involved, toxin production, transportation and fate, environmental conditions, or effects on humans and ecosystems. There is widespread concurrence among scientists, risk assessors, and risk managers, however, that the incidence of CHABs is increasing in spatial and temporal extent in the U.S. and worldwide. The Occurrence Workgroup Report and the chapters describing CHABs in Florida, Nebraska, and New York and the Great Lakes contained in this monograph support the hypothesis of increasing CHAB occurrence. A major impediment to the development of a national database is the lack of a consensus on taxonomy for cyanobacteria. Most field taxonomists rely on the traditional morphology-based botanical approach for phylogenetic classification because molecular data are not available for many species. Research is needed to develop a consensus on taxonomy for cyanobacteria based on genetic fingerprints or an array of characteristics potentially including morphological, molecular, physiological, bioinformatic, and biogeochemical information to classify algal communities with depth and precision.

Nationwide surveys to describe CHAB occurrence will become practical as improved analytical methods to identify species and quantify cyanotoxins become available. Surveys of CHABs in drinking water sources and recreational waters are needed because both types of surface waters present human health risks during CHABs. The EPA has the regulatory authority to implement the Unregulated Contaminant Monitoring Rule (UCMR) that requires a subset of large municipal water utilities to conduct surveys for substances potentially hazardous to human health. Implementation of the UCMR for cyanobacteria and their toxins is not being considered by the EPA at this time because of the need for less expensive, more reliable, accurate, and field-ready analytical methods for quantifying single toxins and multiple analogues. The EPA also could undertake, require, or encourage CHAB monitoring in recreational waters. The BEACH Act, which amends the Clean Water Act, requires EPA to ensure state adoption of recreational water quality standards, revise water quality criteria, publish beach monitoring criteria, and maintain a beach database (EPA 2006). Occurrence

data are a primary requirement for the Agency to make regulatory determinations concerning the development of CHAB regulations or guidelines.

Long-term monitoring is the only method by which trends in CHAB occurrence over time and space can be identified. In addition to identifying changes in CHAB incidence, long-term monitoring programs can provide data needed to assess a variety of issues including CHAB dynamics, environmental interactions, relationships to global climate change, and effects. An understanding of the interactions between cyanotoxins and environmental factors is needed to assess the potential for exposure of human and other biota. Of particular interest is the transportation of cyanotoxins through aerosols, biota, and water, the accumulation and magnification of the toxins in biota and inorganic matrices, and environmental processes through which cyanotoxins are degraded. Only long-term monitoring of CHABs and their toxins in combination with ecological survey data can reveal the cumulative effects of CHABs on ecosystem diversity and population dynamics.

A long-term goal is integration of CHAB monitoring with emerging earth observation systems - the U.S. integrated earth and ocean observing systems (IEOS, IOOS), the Global Oceans Observing System (GOOS) - which culminate in the Global Earth Observing System of Systems (GEOSS; Oceanus 2007). The goal of these observation systems is the routine and continuous delivery of quality controlled data and information on current and future environmental conditions in forms and at rates required by decision makers to address societal goals such as human health protection and ecosystem sustainability. The systems combine remote and *in situ* monitoring data, data management and communication subsystems, and data analysis and modeling components to deliver near real-time and forecasted information to primary users. The combination of *in situ* and remotely sensed data (e.g., aircraft and satellite detection of photopigment type and quantity), and incorporation into U.S. observing systems, will provide a sustainable system for monitoring CHABs and delivering useable information to risk managers.

Forecasts of imminent CHABs will require the development of predictive models that incorporate near real-time data on physical, chemical, and biological conditions at specific locations. As our understanding of CHAB dynamics and environmental interactions increases, it may become possible to not only predict occurrence, but also to predict toxin production, environmental transport, accumulation, and fate. The validation and iterative development of predictive models can be based both on hindcasts derived from datasets not used in model development, and on empirical evidence collected at predicted times and locations. Models that forecast CHABs will provide a window of time for local officials to take risk management

actions such as public notification to prevent exposure or installation of equipment to vertically mix the water column to disrupt bloom formation.

## **CHAB Causes**

CHABs occur in a wide variety of aquatic environments, and the general conditions associated with the initiation of CHABs are known. CHABs require nutrients, particularly nitrogen and phosphorus, and sunlight, and tend to occur in warm, slow moving waters that lack vertical mixing. However, the dynamics of cyanobacterial interactions with environmental factors involved in bloom formation, and the factors that trigger toxin production, are poorly understood. Retrospective analyses of long-term datasets can identify associations between physical, chemical, and biological variations over space and time and the occurrence of CHABs and toxins. Improved understanding of the complex interactions that promote blooms and toxin production will enable the development of hypotheses that can be tested under controlled conditions in laboratory, microcosm, mesocosm, and perhaps field studies. Issues such as the role of trace metals in bloom and toxin production can be addressed most directly through controlled studies. The responses of cyanobacteria to experimentally controlled variables will provide insights into bloom initiation and toxin production that may lead to the development of improved and environmentally benign strategies for controlling CHABs.

Ecosystem monitoring can be used to test hypotheses derived through retrospective data analyses and controlled studies, and to address location-specific issues. The dynamics of CHAB initiation, sustainment, and termination may vary through interactions with location-specific factors. For example, cyanobacteria predators and infectious agents may be or become abundant in some areas, causing relative rapid termination of CHABs. Monitoring may detect CHABs in previously unaffected water bodies as land use practices shift and global climate change raises temperatures and alters hydrologic conditions. A particularly important issue to address through monitoring is threshold levels of environmental factors at which ecosystems undergo long-term phase shifts that promote CHABs and are difficult or impossible to reverse. Only long-term monitoring can reveal trends in the spatial and temporal incidence of CHABs.

The development of mathematical models of CHAB dynamics and interactions with environmental factors will provide a basic framework for relating causative factors to bloom occurrence, toxin production, bloom maintenance and termination. Models of CHAB dynamics and environ-

mental interactions will form integral components of models that ultimately will be developed to predict local CHAB occurrences and the relative efficiency of local control, mitigation, and prevention strategy options.

## Human Health

Information on the human health effects of cyanotoxins is largely limited to characterizations of effects from single, high-level exposures. Many animal studies describe the dose (usually intraperitoneal or oral gavage dosing) of single cyanotoxins that causes lethality in 50% of the animals in a study ( $LD_{50}$ ). Such studies are useful in that they demonstrate that cyanotoxins are among the most potent toxins known, and in identifying the organ system in which failure is the primary cause of death. However, these studies leave many important questions unanswered. They do not address many issues likely to be of importance in human environmental exposures, such as: 1) the relative potency of cyanotoxins through different routes of exposure (i.e., inhalation, dermal absorption, ingestion; 2) the effects of repeated, low-level exposures; 3) the combined effects from exposure to commonly occurring cyanotoxin mixtures (including additive and synergistic effects); and 4) factors that increase or decrease the susceptibility of individuals (and other animal species) to adverse effects from exposure. A combination of well controlled animal studies and both retrospective and prospective epidemiological studies is needed to provide the scientific basis for developing human health risk assessments for exposure to cyanotoxins.

A significant impediment to both animal and human studies is the lack of rapid, reliable, and inexpensive biomarkers of exposure and effect. Analytical methods are needed to quantify multiple cyanotoxin analogues and metabolites in blood and other biological tissues. Protein and DNA adduct measurements may also be useful in characterizing exposure. Accurate characterizations of cyanotoxin exposure present one of the most difficult challenges to human health research. The primary reason is that CHABs often produce several types of cyanotoxins and numerous analogues that vary in toxicologic properties and potencies. Additionally, animal and *in vitro* studies invariably indicate that crude cell extracts are more potent than dose-equivalent quantities of cyanotoxins observed in the cells. The cause of this superpotency may be potentiation of the cyanotoxin modes of actions by other cellular components, or an outcome of exposure to the cyanotoxins and other components not recognized to be toxic.

The characterization of effects from cyanotoxin exposures presents another difficult challenge to human health research. The biological mechanisms through which cyanotoxins cause acute effects may differ from those that cause delayed or chronic effects because different biological systems vary in their ability to repair tissue damage. Cumulative damage may result from repeated exposures in systems with less efficacious repair or compensatory processes. Also, acute effects are more likely to involve direct effects of toxins, whereas chronic effects may result from secondary toxin actions such as triggering an inflammatory response in the immune system. Research is needed to identify biomarkers of cyanotoxin effects in multiple organ systems to characterize the array of effects that may arise from exposure, particularly repeated, low-level exposures. Biomarkers of effect should include biochemical, behavioral, and other indicators of function in all biological systems that may be affected by the direct or indirect actions of cyanotoxins.

Central to the assessment of health risks from cyanotoxin exposure is characterization of toxicokinetic, toxicodynamic, and dose-response relationships in animal models. Toxicokinetic research is needed to characterize toxin uptake through various routes of exposure, the metabolism of the parent compounds and degradation products, distribution of those compounds in tissues, the time-course of toxin retention in tissues, and the pathways of toxin elimination. Toxicodynamic research is needed to describe the modes of action by which the cyanotoxins and metabolites interact with biological tissues to alter physiology and function within affected organ systems. Dose-response research is needed to describe relationships between toxicokinetic parameters of exposure and adverse health outcomes. It is critical to assess relationships in a variety of animal species, to model inter- and intra-species differences, and to validate the ability of the animal models to predict comparable relationships in humans. The inclusion of potentially susceptible subpopulations in the studies, such as fetuses (through *in utero* exposures), the young, and the aged, is needed to reduce scientific uncertainties and improve the accuracy of risk assessments.

Human exposures to cyanotoxins are most likely to occur through contact with recreational waters and drinking water, although the risk for exposure through food consumption is not well characterized. The probability of high-level exposures through water ingestion is less than that for repeated, low-level exposures through recreational or drinking water contact (e.g., ingestion, dermal absorption, inhalation). However, much less is known about the health risks posed by repeated, low-level exposures. Lower level exposures may cause acute illness characterized by non-specific symptoms such as gastro-intestinal distress, skin rashes, respiratory difficulty, and flu-like symptoms. Lower level exposures also may

cause chronic illness in some individuals, such as that reported following the acute-phase of ciguatera seafood poisoning (Palafox and Buenconsejo-Lum 2005). Whereas acute-phase illness is characterized by gastrointestinal and respiratory distress, the chronic-phase is characterized by sustained fatigue, muscle and joint pains, and severe neurologic symptoms that persist indefinitely. Clinical research is needed to describe modes of action in human illness, and to develop therapeutic interventions beyond the current standard-of-care, supportive therapy. Methods are needed to greatly enhance toxin elimination rates, as are toxin antidotes. Other evidence indicates that chronic conditions such as neurodegenerative diseases and delayed illnesses such as cancer may be associated with repeated exposures to cyanotoxins. Both animal and epidemiologic research is needed to characterize the health risks associated with repeated, low-level exposure to cyanotoxins. Retrospective epidemiologic studies may be able to use existing datasets to explore potential linkages between repeated exposures to cyanotoxins and health outcomes. However, prospective epidemiologic studies are needed for more definitive evidence on causal relationships between repeated cyanotoxin exposures and health outcome. The validation of animal models of cyanotoxin exposure-effect relationships also is largely dependent on the availability of epidemiologic data.

Quantitative models are needed to predict dosages of cyanotoxins to which people may be exposed through contact with contaminated water. Both recreational and drinking water contact provides the opportunity for inhalation, ingestion, and dermal exposures to cyanotoxins. The dosage of cyanotoxins depends on the activities in which people are involved, the durations of those activities, and the concentration of cyanotoxins in the water among other factors. Quantitative models that predict the dosages to which people may be exposed based on these factors will assist risk managers in making decisions to ensure that humans are not exposed to dosages that present a health risk.

Quantitative structure-activity relationship (QSAR) models will assist risk assessors by predicting the dosages of cyanotoxins that pose a health risk when no data are available on the cyanotoxin in question, but data on similarly structured molecules are available. For example, few data are available on the effects of repeated dosing with anatoxin-a(s), an organophosphate cyanotoxin that inhibits acetylcholinesterase. However, anatoxin-a(s) is structurally and functionally similar to organophosphate pesticides such as parathion and malathion. It may be possible to develop a QSAR model that predicts the toxicity of anatoxin-a(s) based on the literature describing the toxicity of organophosphate pesticides. A similar approach may be useful in predicting the toxicity of the multiple analogues of cyanotoxins. There are now over 80 known analogues of microcystins.

QSAR models may be able to predict the toxicity of the analogues, obviating the need for extensive toxicity testing for each analogue.

## **Ecosystem Sustainability**

Although most attention and research has focused on the threats to human and domestic animal health, CHABs also disrupt ecosystems by a variety of mechanisms, most of which are poorly characterized. Toxins produced by CHABs have the potential to affect organisms at a variety of trophic levels. Little is known about cyanotoxin impacts on most aquatic biota or the extent to which CHAB toxins transfer between trophic levels. It is frequently noted that CHABs are not grazed by either planktonic or benthic filter feeders. For example, the return of CHAB blooms to some areas of the Great Lakes is hypothesized to have resulted from the introduction of zebra mussels, which graze other algae, but not colonial CHABs, such as *Microcystis*. Grazing inhibition may be due to toxins, the occurrence of CHABs in large mucoid colonies, or the overall unpalatability of many CHABs. A potentially related problem in shallow water bodies undergoing eutrophication and an increasing frequency of CHABs is a persistent shift from a clear to turbid state. Turbid waters are associated with increased populations of disease causing microorganisms, and declines in primary producer populations, including phytoplankton, benthic algae and vascular plants. Research is needed to clarify relationships between CHAB toxins and other stressors, and the development of adverse ecological conditions such as grazing inhibition, turbidization, and disruption of both benthic and plankton food webs.

Freshwater cyanobacteria are often the predominant phylum of plankton in extremely eutrophic waters. Problems caused during CHAB development and maturation include shading and overgrowth of other algae and aquatic vegetation. The huge amounts of organic material produced by CHABs harm ecosystems, even in the absence of toxin production, following bloom senescence. Bloom die-offs result in large amounts of decaying biomass on the benthos that produce hypoxic and anoxic conditions. The lack of dissolved oxygen stresses and kills many benthic dwellers, resulting directly in the loss of benthic biological diversity and weakening the primary producer end of the food web. This impact extends throughout the food web as biota at intermediate and upper levels increasingly lack sufficient nutritional sources. The impacts culminate in the loss of biological diversity at all levels, including the depletion of populations on which humans depend for food sources, recreational activities, and food stock for

the production of other nutritional sources. The loss of biological diversity also may impact ecosystem sustainability by allowing the expansion of less desirable populations, including toxigenic cyanobacteria, due to the lack of competitive forces. This situation is exacerbated when CHABs also produce toxins.

Finally, CHABs generally occur in areas of poor water quality, environments impaired by multiple stressors. CHABs are both a response to the stressors and an additional stressor. The combined effects of the stressors are additive or synergistic, resulting in such extensive ecological changes that the ecosystem may lose its resilience and reach an ecological threshold beyond which it is difficult to return to a more pristine state. An array of studies are needed that focus first on the impacts of CHABs and their toxins on individual components of ecosystems, and then on interactions between the components. This research can be accomplished through a series of culture, microcosms, mesocosms, and ecosystems field studies. An ultimate goal is to develop models that not only provide comprehensive descriptions of current conditions in local areas, but also predict the impacts of future environmental changes, and assess the efficacy of risk management actions.

## **CHAB Prevention**

Changes in land use practices that increase nutrient input into surface waters have been associated with an increased incidence of CHABs in many locations. Nutrient sources include storm water runoff from fertilized and other nutrient rich lands, discharges from sewage treatment plants and large confined animal feeding operations, as well as airborne depositions of nitrogen from organic sources. The development of effective strategies for preventing CHABs requires an understanding of local conditions. For example, CHAB initiation and duration may be dependent on nutrient input in some surface waters, whereas benthic/pelagic coupling or the cycling of nutrients between sediment, biota and the water column may sustain CHABs in eutrophic environments. Research that characterizes nutrient input rates and benthic/pelagic coupling in local areas is needed to develop land and water management plans that will reduce CHAB incidence and promote aquatic ecosystem sustainability.

An increasing incidence of CHABs is an indication of decreasing water quality, usually due to increasing nutrient loads and/or decreasing flow rates. The ultimate driver of decreasing water quality is land use practices. Watershed management plans are increasingly developed and implemented

to improve land use practices such that nutrient input into surface waters are reduced and controlled. Research is needed to develop more efficient and cost-effective methods for reducing non-point source inputs of nutrients and processing point source nutrients in an ecologically sustainable manner.

Watershed management techniques provide a long-term strategy for reducing CHAB incidence and improving water quality. The implementation of water management plans may provide near-term improvements that prevent or terminate CHABs. Increased flow rates and decreased water temperatures improve water quality and reduce the probability of a CHAB occurrence. Where as flow rate and water temperature control are impractical for many surface waters, evidence indicates that artificial destratification of the water column may be a highly efficient means of preventing and terminating CHABs. The use of bubble systems to vertically mix the water column may be effective on a small scale if the density of bubblers is sufficient, but upscaling to large areas is usually impractical. Alternatively, floating solar powered platforms can host pumps that draw in water from above the benthos (to avoid nutrient resuspension from sediment) but below levels at which CHABs occur. The water is discharged at the surface, creating a vertical mixing loop over areas as large as 35 acres. Research is needed to further assess the effectiveness of bubble and pump vertical mixing systems at controlling CHABs, and to identify the mode(s) of action by which vertical mixing inhibits CHABs. Vertical mixing may inhibit CHABs by disrupting cyanobacteria's ability to regulate its position in the water column, or by inducing the dispersion and amplification of microbial colonies that effectively prey upon or infect cyanobacteria. Additional research may reveal other methods by which the competitive forces against cyanobacteria can be increased.

A long-term goal is the development of models that predict the abilities of land and water management techniques to improve water quality and prevent CHABs at specific locations. These models would incorporate area-specific data on CHAB occurrence and dynamics, the physical, chemical, and biological conditions associated with CHABs, and the relative abilities of land and water management techniques to improve those conditions. These models will provide risk managers with powerful tools for developing cost effective strategies to prevent CHABs.

## **CHAB Control & Mitigation**

Environmentally benign methods are needed to terminate CHABs and neutralize cyanotoxins. Algacides such as copper sulfate have been used for

many years to terminate CHABs, and chelated copper compounds have been used in more recent times to extend the period during which algaecidal activity in the water column is retained. However, algaecides, and to a lesser extent algaestats, have several drawbacks. First, algaecides cause cell lysis, resulting in rapid release of cyanotoxins and high concentrations in the water column. High concentrations of cyanotoxins in the water column increase the health risk for humans in recreational waters. High concentrations also may increase the probability of cyanotoxin accumulation in the food web, as well as the probability that drinking water treatment processes will be insufficient to reduce cyanotoxin concentrations to safe levels. Second, algaecides quickly precipitate out of the water column, presenting a health risk to aquatic biota such as benthic invertebrates. The loss of benthic invertebrates disrupts the food chain, eliminates a pathway for phosphorus uptake, and greatly impedes the decomposition of detritus organic matter, each of which threatens ecosystem sustainability. Third, the use of copper algaecides selects for cells tolerant of copper, leading to the development of copper resistant populations. Fourth, algaecides induce rapid bloom collapse, resulting in large biomass deposition on the benthos. Cellular decomposition often depletes dissolved oxygen, thereby producing anoxic or hypoxic conditions that exacerbate the stress already confronting aquatic biota due to high cyanotoxin concentrations. Oxygen depletion also causes the uncoupling of phosphorus from iron oxides in sediment, resulting in resuspension of phosphorus in the water column and increased probability of new CHABs. Research is needed to determine if algaecides and algaestats, as well as other bloom termination techniques such as ultrasound, can be developed that do not have untoward effects on human health and ecosystem sustainability. Research should evaluate the efficacy and ecosystem impacts of vertical mixing techniques, discussed above under prevention, relative to those of algaecidal and other bloom termination techniques.

Research on processes to neutralize cyanotoxins in surface water has met with limited success and may have drawbacks similar to those described above for algaecides. These processes have included the use of compounds such as alum to coagulate or flocculate cyanotoxins, potassium permanganate or titanium dioxide to oxidize cyanotoxins, and electrocoagulation techniques. In addition to problems involving deposition on the benthos, cyanotoxin neutralization techniques are costly, do not inhibit new CHABs, and are impractical for large scale applications. Research on cyanotoxin neutralization techniques may be more applicable to drinking water than surface water treatment.

The need for research on methods to detect and remove cyanobacteria and cyanotoxins during drinking water treatment is particularly great. Evidence indicates that cyanotoxin levels in finished water can be higher than

that in raw water, presumably because water pressure on cells during filtration causes lysis and the release of toxins. Additionally, cyanobacterial taste and odor compounds, particularly geosmin and 2-methylisoborneol (MIB), frequently necessitate that water treatment utilities expend considerable resources to remove these compounds to reduce the frequency of customer complaints. Water utilities commonly rely on the observation of surface scums to detect CHABs, but because CHABs may occur without visible evidence on the surface, they may go undetected at the utilities. Research and development efforts are needed that result in automated, reliable, and inexpensive techniques to detect and quantify cyanobacteria and cyanotoxins in source waters and water treatment systems. Early detection will enable utility managers to implement specialized treatment processes to remove the cells and toxins before they enter the finished water stream.

Many methods have been used in attempts to remove cyanobacteria and cyanotoxins during drinking water treatment, including various oxidation, absorption, coagulation, sedimentation, traditional filtration, and membrane filtration techniques. To date, none of these techniques has proven to be generally effective for intact cell and cell fragment or toxin removal under the variety of conditions that occur during water treatment. The efficacy of cyanotoxin removal techniques depends not only on the concentration and molecular configuration of toxins and analogues, but also on environmental factors such as pH, temperature, and dissolved organic matter concentration, and on processing parameters such as concentrations and contact durations of oxidants and absorbents with the toxins. Research is needed to assess and further develop techniques for cell removal and toxin degradation during drinking water processing that are applicable under a variety of conditions and that maximize cost and benefit ratios. Assessments should also include analyses of treatment process impacts on the production of toxic disinfection byproducts.

Long-term goals include the development of models that predict the effectiveness, cost, and adverse impacts of control and mitigation techniques. For the control of CHABs in surface waters, the models would include factors describing CHAB species and toxin production, physical characteristics of the water body, ecosystem structure and services (including economic benefit), and functional, cost, and outcome characteristics of alternative control options. For the mitigation of cells and toxins entering source water intakes for drinking water processing, the models would predict the degree to which the contaminants would enter the finished water stream without altering treatment processes, and the outcomes, costs, and benefits resulting from the implementation of supplemental treatment options.

## Risk Assessment & Management

Assessments of the risk posed to human health and ecosystem sustainability by toxic substances in the environment have traditionally been done separately. The rationale has been that the analyses will indicate whether the risk is greatest for human health or ecosystem sustainability at the lowest concentration which adversely impacts either humans or the environment, and that regulatory determination will be based on the one that is most susceptible to those impacts. The conceptual flaw to this approach is that it fails to capture the inherent relationships between humans and the environment; ecosystems partially determine human wellbeing, and humans partially determine ecosystem wellbeing. This intimate interconnection is perhaps nowhere clearer than with HABs. The eutrophication of surface waters due to human activities is the single greatest cause of the worldwide increase in the incidence of CHABs, and CHABs have the potential to irrevocably damage ecosystems and to have severe consequences for human health. Research is needed to develop an integrated approach to the assessment of risks posed by CHABs to human health and ecosystem sustainability. An integrated approach to risk assessment and supporting risk analyses has the potential to characterize the total impact of CHABs on society, thereby better informing risk managers as they develop strategies to reduce risks.

The risks posed by CHABs are complex due to the large variety of CHAB species and toxins, the unpredictability of toxin production, the adverse impacts of CHAB biomass independent of toxins, the impacts of CHAB toxins on aquatic biota, and many other issues that have not been sufficiently clarified through research. Research must fully address several issues in order to reduce scientific uncertainties and improve CHAB risk assessments. First, reduced ecosystem sustainability due to lost biological diversity from excess CHAB biomass and toxins must be assessed. Research is needed to better characterize the total impact on CHABs on ecosystem sustainability and the ecological services that are provided to humans.

Second, risk assessments usually identify concentrations at which single toxins do not pose a risk for adverse health effects over a lifetime of exposure. Accordingly, the only World Health Organization (WHO 1999) guideline on cyanotoxins in drinking water is for Microcystin LR, 1  $\mu\text{g/l}$ . That assessment assumed that Microcystin LR is noncarcinogenic, implying that there is a threshold level of exposure below which there is no adverse biological effect. However, there is recent evidence indicating that Microcystin LR may be a carcinogen. The regulatory determination proc-

ess typically incorporates data on the occurrence, dose-response functions, and practical means for reducing risk. Existing evidence has been deemed insufficient to support risk assessments for Microcystin LR and every other cyanotoxin in the U.S. In addition to microcystins, cylindrospermopsins, anatoxins, and to a lesser extent saxitoxins, are considered to be priority cyanotoxins in the U.S. Research is needed to support risk assessments and regulatory determinations for individual cyanotoxins.

Third, the actual risks posed by toxigenic CHABs are not characterized by risk assessments for single cyanotoxins such as Microcystin LR because of several complicating factors. Toxigenic CHABs rarely if ever produce only a single analogue of a single cyanotoxin type. CHABs often produce multiple analogues of a cyanotoxin type, and the analogues may differ in toxic potency. In recognition of this fact, several countries such as Australia have produced guidelines for the total sum of Microcystins in a bloom, 1.3  $\mu\text{g/l}$ . That guideline level is based on the equivalent toxicity of a number of analogues to that of Microcystin LR. In order to produce guidelines for mixtures of cyanotoxin analogues, data are needed that express the toxicity of each analogue to that of a “reference” analogue, as was produced for some of the analogues of Microcystin LR. Toxicity equivalence factors can be derived in two ways: 1) through direct comparisons of the toxicity of each analogue to that of the “reference” analogue as indicated by a bioassay test or; 2) by the development of QSAR models that estimate the relative toxicity of the analogues through an understanding of structure-activity relationships. Therefore, research is needed that supports the development of risk assessments for priority cyanotoxin types based on toxicity equivalence factors.

Fourth, another complicating factor that impedes the characterization of the actual risk posed by toxigenic CHABs is that many blooms contain more than one type of cyanotoxin. Many cyanobacteria species produce several types of cyanotoxins, and some blooms are composed of more than one cyanobacteria genera, each of which may produce different types of cyanotoxins. Yet risk assessment processes have not been developed that can characterize the risk of mixtures of different types of toxins, particularly when the mixtures can vary significantly over time and between locations, as with CHABs. Further complicating the situation is the observation that crude cell extracts are invariably more toxic than the toxins isolated from those cells. Innovative approaches are needed to assess the actual risks posed by mixtures of toxins produced by CHABs.

Fifth, risk assessments are generally designed to be protective of the most vulnerable members of a population. Factors that may render humans more susceptible to toxic exposures include developmental stage (i.e., *in utero*), life stage (e.g., the young and elderly), preexisting illnesses, past

exposures, and concurrent stressors. These and other factors may also pertain to the vulnerability of aquatic biota. Research is needed to better describe factors that increase the susceptibility of humans and other life forms to injury from CHABs.

In addition to the risks posed by CHABs in surface waters, there is need to address the potential risks from cyanotoxin release into other media through accidental or intentional causes. Research on cyanobacteria and cyanotoxins is largely dependent on the production, storage, and transportation of these substances. There is also an increasingly frequent need for transportation of cyanotoxin standard reference materials between suppliers (e.g., commercial and academic) and users (e.g. laboratories, utilities, environmental and health agencies). The need for cyanotoxin-related materials must be balanced with the inherent risk of accidental release of these highly toxic substances. Therefore, cyanobacteria and cyanotoxins are increasingly coming under the regulatory control of international agencies such as the International Air Transport Association (IATA 2006). It is critical that the suppliers and users know of and comply with regulations concerning cyanobacteria and cyanotoxins. On the other hand, it is essential that the regulations are not so cumbersome that they overly inhibit research and risk management processes, thereby increasing the risks from the natural occurrence of these substances. Research is needed to better characterize the risks from accidental release of cyanotoxins during production, storage, and transportation in order to produce regulatory controls that optimize risk reduction.

The potential for weaponization of cyanotoxins has been recognized (USA Patriot Act 2001, ATCSA 2001). Cyanotoxins are high potency and relatively low molecular weight compounds that can be extracted and purified from cultures and in some cases synthesized in laboratories. These features provide the potential for stockpiling large quantities of cyanotoxins for illegitimate purposes. Large populations could be exposed to cyanotoxins through several routes, including municipal water supplies, foodstuffs, and airborne dispersion. Acts as simple as deliberately dumping truckloads of fertilizer into municipal water supplies to trigger CHABs could lead to major societal disruptions, health risks, and economic losses. Yet the risk for cyanotoxins, and other biotoxins, to be used as chemical/biological weapons has not been well characterized. For example, few studies have compared the relative potency of cyanotoxins when delivered through different routes of exposure. Little is known about the potential for preparing cyanotoxins for airborne dispersion, either through the generation of aerosols or production of ultrafine particles from crystallized cyanotoxins. Inhalation exposure to the alkaloid neurotoxins is likely to be much more dangerous than exposure through ingestion due to increased

absorption and rapid access to brain. Research is needed to better characterize the risks and develop risk management strategies to protect against the use of cyanotoxins as weapons.

Quantitative models are needed to estimate the total cost of CHABS on local and national scales to inform regulators and improve risk management strategies. The costs of CHABS can generally be separated into direct costs of CHABS and the costs of CHAB prevention, control, and mitigation. Some direct costs of CHABS may be relatively easy to quantify, such as costs borne by fisheries due to lost productivity, and decreased revenues to merchants from declines in tourism and recreational activities of local populations. Many other direct costs may be difficult to quantify or even go unrecognized. For example, although it might be generally agreed that the costs of CHABS are incurred in categories such as human health and quality of life, ecosystem services, ecological decline, property values, and animal death, it may be difficult to recognize all factors within these categories and to quantify their costs. Innovative approaches are needed to identify all areas in which CHABS have direct impacts and to quantify the costs of those impacts. Assessments of the direct costs of CHABS are needed for comparison with the costs of CHAB prevention, control, and mitigation strategies.

The costs of CHAB prevention, control, and mitigation options can be determined more readily. For example, the costs of implementing watershed management practices or installing vertical mixing apparatus to prevent or control CHABS can be assessed using standard economic approaches. Likewise, costs of mitigation practices such as implementing supplemental water treatment processes or obtaining alternative sources of drinking water can be calculated with reasonable precision. Quantitative models of the direct costs of CHABS and the costs of prevention, control, and mitigation strategies will improve risk management decisions by minimizing costs to the public and maximizing public benefits.

It is critical to measure the efficiency and effectiveness of risk management decisions to provide accountability to risk management systems. As with all governmental initiatives, public support of risk management practices is dependent on the ability to clearly demonstrate that the benefits exceed the costs. Economic models should be developed to quantify the costs of CHABS and CHAB risk management strategies, and to estimate the economic benefits derived from risk management. Application of the models before and after implementation of risk management strategies provides a method for assessing the efficiency and effectiveness of risk management decisions. Efficiency and effectiveness can be maximized over time through an iterative process as improved models and risk management practices are developed.

## Infrastructure

As stated in HARRNESS (2005), “Development of infrastructure will be key to the success of the National Plan, and will ensure that the new strategy is responsive to the needs of scientists, managers, public health coordinators and educators.” The need for infrastructure is also emphasized in the Congressionally mandated, Scientific Assessment of Freshwater Harmful Algal Blooms (FASHAB 2007). These documents and the ISOC-HAB workgroups identify a number of roles for a distributed network of HAB research centers that are crucial in developing a systems approach to assessing and managing the risks posed by CHABs. Central to the concept of CHAB infrastructure is shared facilities that provide high complexity equipment and certified analytical procedures, certified toxin standards and other reference materials, bulk cyanotoxins, database development and management, assistance in the coordination of CHAB research, and educational services. A stable infrastructure will increase the efficiency of CHAB risk assessment and management efforts by reducing redundancies in CHAB research and providing state of the art services to stakeholders.

HAB infrastructure centers ideally would be shared between marine and freshwater research and management communities. The centers would provide the expertise and equipment for culturing and storing cells, extracting and purifying toxins, identifying novel toxins, certifying analytical methods, and validating tests to be used in the field to identify and quantify cells and toxins. The certification of analytical methods might best be done in association with organizations such as the Association of Analytical Communities, International (AOAC 2007). An essential function of the centers would be to provide cell, certified toxin standards, and other reference materials of a known type or degree of purity for use in developing methods and calibrating equipment. Bulk cyanotoxins are needed to characterize the effects of exposure in animal models. The centers should serve as conduits for the integration of many existing databases on cyanobacteria properties, environmental characteristics, and CHAB events for both research purposes and the development of visualization and prediction tools. The databases should be standardized using the IOOS Data Management and Communications (DMAC) structure for reporting and distributing data so that they can be integrated into emerging observation system networks. The integration and standardization of databases for research and model development will yield products that can be utilized by a wide user community. HABHRCA (2004) specifically calls for improved coordination of Federal CHAB research. The FASHAB (2007) report addresses Federal research coordination and recognizes the value of improved coordination

across all levels of U.S. and International CHAB research. The centers could take a lead role in integrating perspectives of the U.S. freshwater HAB research community into the National HAB Committee, a group previously dedicated to the coordination of marine HAB research (HARRNESS 2005). A similar role in international coordination could be taken by the centers through interaction with groups such as CYANONET (2007). The centers also could take the lead role in providing educational services and products to stakeholders and the general public. Educational services could range from the training of volunteers and employees of industry, utilities, state and local governments in the use of equipment to monitor for CHAB cells and toxins to the formal education of students pursuing careers in related fields. Educational products could range from pamphlets and website materials to inform the general public of CHAB risks and management strategies to standardized health advisories for state and local public health officials to issue when CHABs are predicted or in progress. A strong and stable infrastructure provides an efficient foundation to support development of a systems approach toward the assessment and management of CHAB risks.

## **Aligning the Infrastructure and Research Needs with HABHRCA**

The 2004 reauthorization of HABHRCA calls for a “competitive, peer-reviewed, merit-based interagency research program as part of the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB 2007) project, to better understand the causes, characteristics, and impacts of harmful algal blooms in freshwater locations...” The interagency (NOAA, EPA, NSF, NASA, ONR) ECOHAB program and the NOAA Monitoring and Event Response for Harmful Algal Blooms (MERHAB 2007) programs currently meet this requirement. However, the freshwater focus has been on Great Lakes and upper reaches of estuaries because those areas are within the purview of NOAA. No equivalent program exists for inland waters other than the Great Lakes, whereas HABHRCA calls for an examination of the causes, consequences, and economic costs of freshwater HABs throughout the U.S. The causes, characteristics, and impacts of HABs in smaller freshwater bodies are likely to differ significantly from those of marine and Great Lakes HABs. In addition, research related to HABs in drinking source waters and treatment options, an important public health issue, falls out of the scope of the programs mentioned above. Finally, although there

are programs that provide assistance to state and local management agencies in responding to marine HABs to protect human health and coastal economies, there are no such programs for inland freshwater HABs. Thus, state, local, and tribal governments are left to develop management strategies without an adequate scientific basis or Federal guidance. This problem could be solved either by broadening the scope of existing programs or establishing programs specifically for freshwater/inland HABs within agencies having the appropriate mandate.

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