1. **Since the release of the initial plan last November, what steps have been completed, what progress has been made and knowledge gained, and what is currently planned for:**

   a. *Identifying gaps in the agency's understanding of algal toxins. Selected progress includes the following activities:*
      - As the EPA progresses with the implementation of the Strategic Plan and learns from various research efforts, the agency is tracking any research gaps identified by the emerging science.
      - The EPA has also gained additional insights on information gaps through three Regional HABs source water protection workshops held in late 2015 and 2016. These workshops provide a unique opportunity to identify major issues surrounding prevention and mitigation of harmful algal blooms and management of cyanotoxins at the state and local level.
      - The EPA has gained additional insights on gaps in information for adequately managing cyanotoxin risks in drinking water during our April 2016 public meeting.

   b. *Evaluating the risk to human health from drinking water contaminated with algal toxins. Selected progress includes the following activities:*
      - Understanding human health effects of algal toxins and the factors that cause the bacteria to proliferate and produce toxins is a priority for the EPA. In 2015, the EPA released Health Advisories for microcystins and cylindrospermopsin. Health Advisories are non-regulatory values that serve as informal technical guidance to assist federal, state and local officials, and managers of public water systems to protect public health from contaminants. In 2015, the EPA also released Health Effects Support Documents for microcystins, cylindrospermopsin and anatoxin-a, which describe health effect information for each of the specific toxins. It was determined at that time that there was not sufficient health effects information to develop a Health Advisory for anatoxin-a, and the EPA continues to evaluate new information on anatoxin-a as it becomes available.
      - The EPA is currently developing Recreational Ambient Water Quality Criteria for microcystins and cylindrospermopsin and anticipates releasing a draft criteria in 2016.
      - The EPA is also re-evaluating the current CWA section 304(a) nationally recommended numeric nutrient criteria for lakes and reservoirs and is considering proposing values later this year to specifically protect drinking water and recreational water for HABs development.
      - For additional activities on evaluating human health please see *Section 2.d* of this summary.

   c. *Establishing, publishing, and updating a comprehensive list of algal toxins that may have an adverse effect on human health, taking into account likely exposure levels. Selected progress includes the following activities:*
      - The EPA included cyanotoxins on the draft fourth Contaminant Candidate List (CCL4). The CCL is a list of contaminants that are currently not subject to any proposed or promulgated national primary drinking water regulations, are known or anticipated to occur in public water systems and that may require future regulation under the Safe Drinking Water Act (SDWA). The EPA identified specific cyanotoxins as being potentially of concern. The EPA is currently evaluating comments received on the draft list, and anticipates publishing the final CCL4 in 2017.

   d. *Summarizing the known adverse human health effects of algal toxins and the factors that cause toxin-producing cyanobacteria and algae to grow rapidly and make toxins. Selected progress includes the following activities:*
      - The EPA continues to evaluate new toxicity peer reviewed studies to update the Health Effects Support Documents and Health Advisories as appropriate.
      - In addition to the agency's progress on evaluating human health impacts from harmful algal blooms in public water supplies (discussed under *Section 1.b* of this summary), in the Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water the EPA

identified multiple factors that could contribute to the proliferation of blooms and production of toxins, including nutrients (nitrogen and phosphorus), increased rainfall events, warmer temperatures, and land use surrounding sources of drinking water.

- The EPA has ongoing research initiatives to develop modeling tools to better understand factors of bloom and toxin production, such as the Regional Applied Research Effort. Cyanotoxins and other water quality parameters continue to be part of the National Aquatic Resource Surveys that provide data for analysis to better understand water quality parameters such as nutrient levels and their impacts on cyanotoxin occurrence. The most recent National Lakes Assessment data are anticipated to be released in 2016.
- The EPA is collaborating with the National Oceanic and Atmospheric Administration focusing on research in the Great Lakes to develop systematic approaches to evaluate existing indicators and develop new indicators of the occurrence of HABs and their toxins. This research also includes evaluating patterns and trends in bloom and toxin development in the Great Lakes and other lakes and estuaries.
- For additional activities on evaluating factors leading to HABs and toxin production see *Section 2.b* of this summary.

e. *Establishing guidance regarding feasible analytical methods to quantify the presence of algal toxins and guidance regarding the frequency of monitoring necessary to determine if the algal toxins are present. Selected progress includes the following activities:*
    - The EPA provided analytical method and monitoring recommendations to drinking water utilities by including an overview of available analytical methods in the June 2015 Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water*.* A discussion on available analytical methods is also provided on the EPA's HABs website (https://www.epa.gov/cyanohabs). The EPA continues to engage in new method development, optimizing current methods, and increasing laboratory capacity. The EPA published two methods for cyanotoxin analysis in drinking water, the liquid chromatography tandem mass spectrometry (LC/MS/MS) methods: EPA Method 544 (February 2015) for determination of select microcystins and nodularin-R and EPA Method 545 (April 2015) for determination of anatoxin-a and cylindrospermopsin. The EPA is working on an ADDA Enzyme-Linked Immunosorbent Assay (ELISA) method for microcystins in support of the fourth Unregulated Contaminant Monitoring Rule (UCMR 4).
    - The EPA is developing monitoring protocols and technologies using remote satellite sensing techniques. The EPA continues to work collaboratively with the National Aeronautics and Space Administration, NOAA, and the United States Geological Survey on the Cyanobacteria Assessment Network (CyAN) to detect and quantify cyanobacterial blooms in U.S. freshwater lakes and reservoirs using satellites.
    - For additional activities on analytical methods and monitoring please see *Section 2.a* of this summary.

f. *Recommending feasible treatment options, including procedures, equipment, and source water protection practices. Selected progress includes the following activities:*
    - The EPA continues to provide information on feasible treatment techniques, nutrient reduction strategies, and source water protection practices. The EPA recommended both treatment techniques and source water protection activities in the Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water*.*
    - The EPA is working with drinking water systems to identify specific protocols to optimize the drinking water treatment process to remove cyanotoxins through the Area-Wide Optimization Program (AWOP).

- The EPA is working to develop tools to assist with source water protection activities including releasing in February 2016 the Drinking Water Mapping Application to Protect Source Waters (DWMAPS), which can provide public water systems, source water collaboratives and watershed groups, and other users with information to better understand sources of pollution near drinking water sources and prioritize source water protection measures.
- The EPA, along with many partners, launched the Nutrient Recycling Challenge in November 2015 to promote development of affordable technologies to recycle nutrients from livestock manure. This challenge can help develop technologies that could help reduce nutrients reaching sources of drinking water. The winners of the challenge were announced in March 2016.
- The EPA is hosting HABs source water protection workshops, focused on harmful algal blooms and cyanotoxins, in all EPA regional offices. To date, three workshops have been held in Region 8 (September 2015), Region 10 (March 2016), and Region 5 (April 2016). Planning is underway for additional workshops in 2016 and 2017.
- The EPA continues to be actively engaged in the Source Water Collaborative, a group made up of 26 national organizations, whose goal is to protect America's drinking water at the source.
- For additional activities on toxin removal through drinking water treatment see *Section 2.e* of this summary.

g. *Entering into cooperative agreements with, and provide technical assistance to, affected states and public water systems to manage risks associated with algal toxins. Selected progress includes the following activities:*
   - The EPA continues to use cooperative agreements and provide technical support to states and public water systems through building and maintaining relationships, capacity building and technical assistance, funding opportunities, outreach and communication activities, and workgroup involvement.
   - Examples of these activities include engaging with the Association of State Drinking Water Administrators (ASDWA) to obtain support and input on ongoing activities, such as the development of cyanotoxin management plan templates. The EPA is working with five utilities in four states to develop system-specific cyanotoxin management plans in order to provide cyanotoxin management plan templates for a variety of system types. These templates can provide nationwide examples for systems to develop their own drinking water cyanotoxin management plans as they deem appropriate.
   - Other examples of EPA partnerships includes the Region 5 Great Lakes Restoration Initiative (GLRI), the National HABs Committee, and the Interagency Working Group (IWG) initiated by the Harmful Algal Bloom and Hypoxia Research and Control Act. The IWG released in February 2016 the Harmful Algal Blooms and Hypoxia Comprehensive Research Plan and Action Strategy: An Interagency Report.
   - The EPA collaborated with Centers for Disease Control and Prevention in developing the One Health Harmful Algal Bloom System (OHHABS) to collect data on harmful algal blooms and associated human and animal illness. OHHABS, launched on June 22, 2016, will support health officials and the public in managing the risk of HABs and their subsequent health impacts.

2. In Appendix 3 of the Agency's November 2015 Strategic Plan for Algal Toxin Assessment, the Agency mentioned its work on Reducing Impacts of Harmful Algal Blooms (HAB), for its 2016-2019 research cycle. What progress has been made as part of this effort to:

a. *Improve the science of HAB and toxin detection by developing HAB-specific analytical methods and sampling strategies. Selected progress includes the following activities:*
   - Method 544 for microcystins (6 congeners) and nodularin in drinking water completed and posted on-line. This method is proposed in support of analyses for the UCMR 4.

- Analytical methods for microcystins (13 congeners)/nodularin and anatoxin-a/ cylindrospermopsin in ambient waters are under review.
- An enzyme-linked immunosorbent assay (ELISA) method has been developed by the EPA for UCMR 4. EPA is currently performing a multi-laboratory validation of the method and is targeting the end of June 2016 for completion of the validation process.
- The EPA is currently developing an additional liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for microcystin congeners. The LC-MS-MS method targets a specific molecular portion, common to most microcystin congeners: 2-methoxy,3-methyl,4-phenyl butyric acid (MMPB). Studies are underway in ambient water that compare the MMPB/LC-MS-MS method with ELISA and solid phase extraction LC-MS-MS (15 congeners) methods.
- The EPA is currently developing methods for analyzing fish tissues for cyanotoxins.
- The EPA is developing molecular methods to simultaneously determine the occurrence and relative abundance of the relevant toxin-producing algal species and the genes linked to toxin production.

b. *Assist the National Water Program in developing new HAB indicators, sampling designs, and protocols for use in national-scale assessments. Selected progress includes the following activities:*
   - The CyAN Project is a collaborative effort among the EPA, NASA, NOAA, and USGS to provide an approach for integrating satellite data with water management decisions. The project officially began on October 1, 2015, and initially focused on eight states. Plans are underway to expand the project over the entire continent.
   - Studies comparing/contrasting satellite reflectance algorithms for estimating chlorophyll-a using aircraft imagery and surface water observations are complete. Several algorithm/satellite imagery combinations were identified for future use on inland water bodies.
   - Analysis completed on the EPA's nation-wide survey data of cyanotoxins in U.S. lakes (1,161 lakes) that includes cyanotoxin concentrations and assessment of chlorophyll as an indicator.
   - Analysis completed on using phycocyanin as an indicator for the presence of cyanotoxins. In addition to fundamental studies comparing measured phycocyanin and cyanotoxin concentrations, EPA researchers are employing spectrophotometry coupled with fluorescence microscopy to obtain spectral images of lake water to identify unique phycocyanin spectra from various species of cyanobacteria and algae. Preliminary research results suggest that cyanobacteria and algae under stress exhibit different spectral patterns that may be used as indicators for imminent toxin production.
   - The EPA is developing a smart-device application for analyzing photographic images for crowdsourcing the detection and identification of cyanobacterial/algal blooms in lakes and rivers. The application differentiates colors at the individual pixel level to estimate the prevalence of cyanobacteria at any given time.

c. *Develop improved approaches to understanding the interactive effects of increasing water temperatures and nutrient loads on HAB development and toxin production/Develop improved models to project risk of HABs under warming climate scenarios. Selected progress includes the following activities:*
   - Research efforts have focused on building and improving water quality models for predicting the transport of nitrogen and phosphorous into receiving waters to assess the environmental consequences of these nutrients.
     - Published paper on random forest modeling efforts relating nutrients to lake trophic state, which is believed to be strongly associated with cyanobacteria.
     - Published paper on microcystin-LR toxin and chlorophyll-a associations, concluding that when managing for these various microcystin levels, exceeding specified reported chlorophyll-a concentrations should be a trigger for further testing and possible management action.

    - Researchers have developed and presented on Bayesian models of toxin presence absence and surface water trophic state.
- Initiation of a modeling effort through the EPA Office of Environmental Information's Digital Analytics Program (E-DAP), providing a scalable data, analytics and visualization platform for the agency to forecast HABs.
- The EPA developed the Water Quality Assessment Tool (WQAT) for satellite data. This tool provides simplified access to remote sensing imagery of indicators of nutrient pollution. WQAT is targeted as a niche tool for water quality management of nutrient pollution, water clarity, and suspended sediments, and is being used as a nutrient management tool to provide technical assistance to the states.
- The EPA is also developing new approaches to understand the interactive effects of increasing nutrient loads on HAB development and toxin production, and is engaging state partners in a review of these approaches.

d. *Improve understanding of the human health and ecosystem effects resulting from toxin exposure. Selected progress includes the following activities:*
- A 90-day toxicity study of cylindrospermopsin has been completed. This study will enable the EPA to refine Tolerated Daily Intake safety guidelines for the toxin in drinking water.
- The EPA is currently evaluating the use of non-invasive tissue samples from humans to assess exposures to toxins in HABs.
- The EPA is currently studying the comparative toxicities of six common chemical variants in the microcystin group (MCLR, MCRR, MCYR, MCLY, MCLA, MCLF). This data will increase the ability of the EPA to evaluate potential adverse health effects of mixtures of these toxins.
- Studies are ongoing to determine the mechanism of the bleeding that the toxin cylindrospermopsin induces. This understanding may assist development of treatment strategies in human exposure cases.
- The EPA developed a critical review (not yet published) on the role of beta-N-methylamino-L-alanine (BMAA), a compound produced by many cyanobacteria, in neurological diseases in humans. The review concludes that existing data do not support assumptions of a causal relationship between BMAA and the neurological diseases and further research is needed.
- The EPA is currently performing research on the biological effects from different microcystin congeners on human colonocytes. Based on the observed results, this may be followed by experiments with hepatocytes and renal cells.
- Studies are underway on characterizing cytotoxicity, cyanotoxin transport across cell membranes, metabolism, formation of reactive oxygen species, and activation of pathways involved with cancer.
- The EPA is investigating the oral bioavailability and dermal absorption of microcystin-LR in experimental models.
- The EPA is currently investigating the role of cyanobacteria and cyanotoxins in prompting allergic reactions after human exposure through recreational activities. Research was recently published on cyanobacteria effects on recreational beachgoers.
- EPA scientists published research on the effects of microcystin-LR on placental/fetal development. One of the main conclusions was that microcystin-LR doses below the cytotoxic dose of 25 micro moles did not affect placental cell viability but changed placental cell function.
- The EPA recently published research on the risk of cyanotoxins in contaminated dialysis water.
- The EPA reviewed reports of illnesses associated with exposure to cyanobacteria and found that animal illnesses and deaths may be sentinel events for recognizing human health risks.

e. *Provide drinking water treatment system operators with improved methods for detecting and treating toxins in order to limit or prevent human exposures. Selected progress includes the following activities:*

- The EPA is currently scaling-up bench-scale studies that evaluated the impacts of potassium permanganate (permanganate) and powdered activated carbon (PAC) additions on cyanotoxin removal in drinking water treatment. Two pilot-scale systems have been designed and are planned to be installed in the City of Toledo's low service pumping station this bloom season. These pilot units are designed to simulate contact times for permanganate and PAC. Coagulation, softening, flocculation and settling processes are also being assessed. Results from these pilot plant studies will be disseminated nationally and used to optimize drinking water treatment strategies for cyanotoxins.
- The EPA is following up on summer 2015 studies at nine drinking water intakes in Region 8 with 2016 studies at nine additional intakes in Region 8 to evaluate the presence of microcystins and cylindrospermopsin at drinking water treatment facility intakes. By the end of the 2016 bloom season, sampling will have been performed in Colorado, Montana, Utah and Wyoming.

3. What is the role of stakeholders (including States and drinking water systems) in working with the Agency to improve scientific information and tools to assess, predict, and manage the risk of HABs, associated toxicity events, and the ensuing ecological, economic and health impacts?

Selected examples of strong collaboration with stakeholders to make progress on Strategic Plan goals include the following activities:

- On April 29, 2016, the EPA held a public meeting to seek feedback on strategies for managing cyanotoxin risks to drinking water. Approximately 180 stakeholders including local and state governments, industry and industry representatives, academia and public water systems provided valuable feedback.
- The EPA continues to partner with local and state governments, drinking water utilities representatives, and public water systems to develop cyanotoxin support tools, which can be used by utilities in developing monitoring, treatment and communication plans for addressing cyanotoxins in their drinking water. Some of these tools will be completed in 2016.
- The EPA is partnering with states to sponsor workshops that focus on source water protection practices to prevent and reduce cyanotoxin occurrence at the state and local level. This partnership has been invaluable for: identifying current gaps in scientific understanding, identifying needed implementation tools and exploring best practices for source water protection strategies to prevent HABs. Three workshops have been held since fall 2015, and planning is underway for additional workshops in 2016 and 2017.
- In order to help disseminate information to the public on the risks from cyanotoxins, the EPA is developing a risk communication package for public water systems to use in their communication with the public. The EPA is working closely with a variety of stakeholders, including states, drinking water utilities representatives, non-profit research organizations and other federal agencies, to ensure the messaging is easy to understand and consistent. These materials are anticipated to be released in 2016.
- The EPA is also partnering with states by asking for their review of the EPA's new approaches to understanding the interactive effects of increasing nutrient loads on HAB development and toxin production.
- Additionally, examples of the EPA's collaboration with key federal stakeholders include: participating with NOAA, the CDC, the Food and Drug Administration, the U.S. Department of Agriculture, the U.S. Army Corps of Engineers, the U.S. Geological Survey (USGS), NASA, the National Institutes of Health, and the National Science Foundation as part of the Harmful Algal Bloom and Hypoxia Research and Control Act Interagency Working Group, co-chaired by the EPA and NOAA, and collaborating with CDC in developing the One Health Harmful Algal Bloom System (OHHABS) to collect data on HABs and associated human and animal illness.

Key recent publications on cyanobacteria and HABs authored and coauthored by EPA staff

Aron, J., R. Hall , B. Niemann, D. Heggem , R. Schafer, and J. Lin. Mining Datasets to Facilitate the Use of Ecosystem Function in the Clean Water Act: Case Study of the Control of Freshwater Harmful Algal Blooms in Oregon. Submitted to the *Journal of Policy Analysis and Management.*

Oregon provides a case study of a statewide approach with a HAB monitoring program and an inventory of voluntary watershed restoration projects. Using a metric of riparian miles affected for these restoration projects, Big Data techniques identified the top four basins as Southern Oregon Coast, Northern Oregon Coast, Willamette and Lower Columbia River. In the Southern Oregon Coast basin, Tenmile Lakes Watershed is the most heavily affected by freshwater HABs. Tenmile Lakes Watershed provides an example of local detailed information required to link watershed restoration decisions to NPS pollution control and HAB control under the Clean Water Act. Future work should address implementation in Oregon and extension to other states as well as the improvement of environmental tools and supporting datasets.

Abdelrhman, M. Modeling light attenuation and backscattering to promote the recovery of submerged aquatic vegetation (SAV) in Narragansett Bay, Rhode Island. Submitted to the journal *Estuarine Coastal and Shelf Science*.

The availability of adequate light intensity is a major factor in the health of algae and seagrass beds in estuarine and coastal systems. The concentration of total suspended solids, including phytoplankton, in the water column controls water clarity and the amount of light that penetrates to any depth as well as the irradiance near the bed for submerged aquatic vegetation (SAV). The three dimensional hydrodynamic and water quality model (The Environmental Fluid Dynamics Code, EFDC) is used to estimate concentrations of chlorophyll-a (Chl-a) in the suspended particulate organic matter which encompassed three groups of phytoplankton: cyanobacteria, diatoms, and green algae. The Chl-a concentration estimates are used as a surrogate for light attenuation and backscattering factors in the development of the mathematical methodology to estimate reduction in light intensity in Narragansett Bay, Rhode Island, USA, due to absorption by pure water, phytoplankton pigment, total suspended solids (TSS), and colored dissolved organic matter (CDOM), as well as backscattering by pure seawater, phytoplankton particles, and the other non-algal particles. The methods developed can be used to study the effect of various environmental and management scenarios on the recovery efforts for SAV beds in estuarine and coastal systems.

Beck, R., S. Zhan, H. Liu, S. Tong, B. Yang, M. Xu, Z. Ye, Y. Huang, S. Shu, K. Berling, M. Andrew, E. Emery, M. Reif, J. Harwood, J. Young, C. Nietch , D. Macke , M. Martin, G. Stillings, R. Stumpf, and H. Su (2016). Comparison of satellite reflectance algorithms for estimating chlorophyll-a in a temperate reservoir using coincident hyperspectral aircraft imagery and dense coincident surface observations. *Remote Sensing of Environment*, 178: 15–30.

The authors analyzed 10 established and 4 new satellite reflectance algorithms for estimating chlorophyll-a (Chl-a) in a temperate reservoir in southwest Ohio, using coincident hyperspectral aircraft imagery and dense water truth collected within one hour of image acquisition to develop simple proxies for algal blooms and to facilitate portability between multispectral satellite imagers for regional algal bloom monitoring. Narrow band hyperspectral aircraft images were upscaled spectrally and spatially to simulate 5 current and near future satellite imaging systems. Established and new Chl-a algorithms were then applied to the synthetic satellite images and then compared to calibrated Chl-a water truth measurements collected from 44 sites within one hour of aircraft acquisition of the imagery. Masks based on the spatial resolution of the synthetic satellite imagery were then applied to eliminate mixed pixels including vegetated shorelines. Medium-resolution Landsat and finer resolution data were evaluated against 29 coincident water truth sites. Coarse-resolution

MODIS and MERIS-like data were evaluated against 9 coincident water truth sites. Each synthetic satellite data set was then evaluated for the performance of a variety of spectrally appropriate algorithms with regard to the estimation of Chl-a concentrations against the water truth data set. The goal is to inform water resource decisions on the appropriate satellite data acquisition and processing for the estimation of Chl-a at the water surface for near-real-time monitoring of algal blooms in temperate inland reservoirs. The authors found several promising algorithm/satellite imager combinations for routine Chl-a estimation in smaller inland water bodies with operational and near-future satellite systems.

Brooks, B., J. Lazorchak, M. Howard, M. Johnson, S.L. Morton, D.A.K. Perkins, E.D. Reavie, G.I. Scott, S.A. Smith, and J.A. Steevens (2015). Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental Toxicology and Chemistry*, 35(1): 6-13.

In this Focus article, the authors ask a seemingly simple question: Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? When HAB events require restrictions on fisheries, recreation, and drinking water uses of inland water bodies significant economic consequences result. Unfortunately, the magnitude, frequency, and duration of HABs in inland waters are poorly understood across spatiotemporal scales and differentially engaged among states, tribes, and territories. Harmful algal bloom impacts are not as predictable as those from conventional chemical contaminants, for which water quality assessment and management programs were primarily developed, because interactions among multiple natural and anthropogenic factors determine the likelihood and severity to which a HAB will occur in a specific water body. These forcing factors can also affect toxin production. Beyond site-specific water quality degradation caused directly by HABs, the presence of HAB toxins can negatively influence routine surface water quality monitoring, assessment, and management practices. Harmful algal blooms present significant challenges for achieving water quality protection and restoration goals when these toxins confound interpretation of monitoring results and environmental quality standards implementation efforts for other chemicals and stressors. Whether HABs presently represent the greatest threat to inland water quality is debatable, though in inland waters of developed countries they typically cause more severe acute impacts to environmental quality than conventional chemical contamination events. The authors identify several timely research needs. Environmental toxicology, environmental chemistry, and risk-assessment expertise must interface with ecologists, engineers, and public health practitioners to engage the complexities of HAB assessment and management, to address the forcing factors for HAB formation, and to reduce the threats posed to inland surface water quality.

Brill, J., S. Belanger, J. Chaney, S. Dyer, S. Raimondo, M. Barron , and C. Pittinger (2016). Development of Algal Interspecies Correlation Estimation (ICE) Models for Chemical Hazard Assessment. *Environmental Toxicology and Chemistry,* doi:10.1002/etc.3375.

Web-ICE (Web-based Interspecies Correlation Estimation) is an Internet application developed by the EPA to predict the acute toxicity of a chemical from one species to another taxon. An ICE model uses the acute toxicity value for a surrogate species and predicts the effect value for many other species, thus potentially filling in data gaps for a variety of environmental assessment purposes. Web-ICE has historically been dominated by animal (aquatic invertebrates and fish, terrestrial mammals and birds) prediction models. Toxicity estimation for algal species in Web-ICE was essentially absent and is now addressed in this research. A compilation of public (ECOTOX and scientific literature) and private sector-held algal toxicity data were synthesized and verification of toxicity data values was assessed followed by an extensive review of relevant aspects of individual studies. Interspecies correlations were constructed from the most commonly tested algal genera for a broad spectrum of chemicals. ICE regressions were developed from the final database based on acute 72 and 96 hour ErC50, EbC50 and EC50 values. The final database consisted of 1647 unique studies with 476 unique chemicals and encompassed 40 genera and 70 species of green algae (Chlorophyta), blue-green algae (photosynthetic

Cyanobacteria) and diatoms (Bacillariophyta). Acceptance criteria for algal-ICE models were established prior to evaluation of individual models and included a minimum sample size of 3, statistically significant regression slope, and a slope estimation parameter ≥0.65. A total of 186 ICE models were possible at the genus level with 21 meeting quality criteria and 264 ICE models were developed at the species level with 32 meeting quality criteria. When the minimum sample size was increased to 7, 32 models met significance, slope, and sample size criteria and were considered the most robust. Algal-ICE models will have broad utility in screening environmental hazard assessments, data gap filling in certain regulatory scenarios and as supplemental information to derive species sensitivity distributions. In concert with existing Web-ICE models, new algal-ICE models may assist hazard assessors to be less dependent on animal tests as well.

Bullerjahn, G., McKay, R., Davis, T., Baker, D., Boyer, G., D'Anglada, L., Doucette, G., Ho, J., Irwin, E., Kling, C, Kudela, R., Kurmayer, R., Michalak, A., Ortiz, J., Otten, T., Paerl, H., Qin, B., Sohngen, B., Stumpf, R., Visser, P., Wilhelm, S. et al. (2016). Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. Harmful Algae, Vol 54, pages. 223-238.

Chernoff, N., D. Diggs, D. Hill, J. Schmid, W. Winnik, B. Faison, B. Francis, M. Larue, T. Le, K. Loftin, and J. Lugo. A Critical Review of the Postulated Role of the Cyanobacterial Metabolite, Beta-N-Methylamino-L-Alanine (BMAA) in Neurodegenerative Disease in Humans. Submitted to the *Journal of Toxicology and Environmental Health*.

The compound BMAA (β-N-methylamino-L-alanine) has been hypothesized to play a significant role in four serious neurological diseases in humans: Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PDC) found on Guam, and ALS, parkinsonism, and dementia that occur globally. ALS/PDC with symptoms of all three diseases first came to the attention of the scientific community during and after World War II. It was initially associated with cycad flour used for food since BMAA is a product of symbiotic cycad root-dwelling cyanobacteria. Human consumption of flying foxes that fed on cycad seeds was later suggested as a source of BMAA on Guam and a cause of ALS/PDC. Subsequently, the hypothesis was expanded to include a causative role in the other neurodegenerative diseases through exposures attributed to proximity to freshwaters and/or consumption of seafood due to its purported production by most species of cyanobacteria. The hypothesis that BMAA is the critical factor in the genesis of these neurodegenerative diseases has understandably received considerable attention in the medical, scientific, and public arenas. This review examines the history of ALS/PDC and the BMAA-human disease hypothesis; the similarities and differences between ALS/PDC and the other diseases with similar symptomologies; inconsistencies and data gaps in the hypothesis; and other compounds and agents that have been suggested as the cause of ALS/PDC on Guam. The conclusion of this review is that the assumption of a causal relationship of BMAA to neurodegenerative disease is not supported by the existing data.

D'Anglada, L., Elizabeth Hilborn, E., Backer, L. (2016). Harmful Algal Blooms (HABs) and Public Health: Progress and Current Challenges, 316 pages.

Delacruz, A., N. Chernoff , J. Sinclair , D. Hill , D. Diggs , and A. Lynch. Chapter 1: Introduction to Cyanobacteria and Cyanotoxins. Submitted as a book chapter in *Treatment and Purification of Water Contaminated from Cyanobacteria and Cyanotoxins: Progress, State of the Art, and Challenges*.

Source water for drinking water will contain a host of living microorganisms often referred to as consortia. This consortia consist of viruses, bacteria, fungi, protozoa, and algae. The focus of this publication will concentrate on one group of bacteria, cyanobacteria, but it is important to emphasize that in nature, cyanobacteria are never occurring alone or as one species. The complexities of the interactions between the variety of organisms and their environment will strongly influence the challenges for water treatment and purification when dealing with cyanobacteria. Management of water resources require concerted efforts of public health officials, water treatment managers, scientists and the consumers to protect human health and the environment for future generations.

Douglas, G., T. Thirkill, P. Kumar, M. Loi, and E. Hilborn (2016). Effect of Microcystin-LR on human placental villous trophoblast differentiation in vitro. *Environmental Toxicolog*y, 31(4): 427-39.

Microcystin-LR is a cyanobacterial toxin found in surface and recreational waters that inhibits protein phosphatases and may disrupt the cytoskeleton. Microcystins induce apoptosis in hepatocytes at ≤2.0 µM. Nothing is known about the effects of microcystins on human placental trophoblast differentiation and function. The differentiation of villous trophoblasts to form syncytiotrophoblast occurs throughout pregnancy and is essential for normal placental and fetal development. To investigate the effects of microcystin, villous cytotrophoblasts were isolated from term placentas using an established method and exposed to microcystin-LR. Microcystin-LR below the cytotoxic dose of 25 µM did not cause cell rounding or detachment, had no effect on apoptosis, and no effect on the morphological differentiation of mononucleated cytotrophoblasts to multinucleated syncytiotrophoblast. However, secretion of human chorionic gonadotropin (hCG) increased in a microcystin-LR dose-dependent manner. When incubated with l-buthioninesulphoximine (BSO) to deplete glutathione levels, trophoblast morphological differentiation proceeded normally in the presence of microcystin-LR. Microcystin-LR did not disrupt the trophoblast microtubule cytoskeleton, which is known to play a role in trophoblast differentiation. Immunofluorescence studies showed that trophoblasts express organic anion transport protein 1B3 (OATP1B3), a known microcystin transport protein. In comparison to hepatocytes, trophoblasts appear to be more resistant to the toxic effects of microcystin-LR. The physiological implications of increased hCG secretion in response to microcystin-LR exposure remain to be determined.

Han, C. , J. Lalley, D. Namboodiri, K. Cromer, and Nadagouda, M. (2016). Titanium dioxide-based antibacterial surfaces for water treatment. *Current Opinion in Chemical Engineering*. 11: 46–51.

The field of water disinfection is gaining much interest since waterborne diseases caused by pathogenic microorganisms directly endanger human health. Antibacterial surfaces offer a new, ecofriendly technique to reduce the harmful disinfection byproducts that form in medical and food processing industries. TiO_2 photocatalysts have been extensively studied to prepare antibacterial surfaces due to their environmentally favorable properties. The studies demonstrate TiO_2 improves the efficiency of disinfection by the effective inactivation of pathogenic microorganisms (i.e., *E. coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas putida*, and *Listeria innocua*). TiO_2 photocatalysts decompose natural algal toxins such as microcystin-LR and cylindrospermopsin under solar/visible light irradiation. Based on literature review, these antibacterial surfaces may be applied to hospital, food, ceramic, and building industry or to environmental remediation where bacteria inactivation is required to ensure the safety of human health and the environment.

Hilborn, E., and R. Ward (2016). The Risk of Cyanobacterial Toxins in Dialysate, What do we Know? *Seminars in Dialysis*, 29(1): 15-18.

Surface waters are increasingly contaminated by cyanobacteria, which may produce potent cyanotoxins harmful to animals and humans. Hemodialysis patients are at high risk of injury from waterborne contaminants in the water used to prepare dialysate. Episodes of acute illness and death among hemodialysis patients have been reported following exposure to dialysate prepared from drinking water contaminated with elevated concentrations of cyanotoxins. Protecting dialysis patients from these toxins is complicated by a lack of monitoring and regulation of cyanotoxins in drinking water, uncertainty as to their safe levels in dialysate, and incomplete knowledge of how well current dialysate preparation and water treatment practices remove them.

Until these issues are adequately addressed, hemodialysis centers should be aware of the potential for cyanotoxins to be present in their potable water supply, particularly when that water comes from surface water sources prone to cyanobacterial blooms.

Hilborn, ED and Beasley, VR. One Health and Cyanobacteria in Freshwater Systems: Animal Illnesses and Deaths Are Sentinel Events for Human Health Risks. Toxins 2015. 7(4):1374-1395.

Harmful cyanobacterial blooms have adversely impacted human and animal health for thousands of years. Recently, the health impacts of harmful cyanobacteria blooms are becoming more frequently detected and reported. However, reports of human and animal illnesses or deaths associated with harmful cyanobacteria blooms tend to be investigated and reported separately. Consequently, professionals working in human or in animal health do not always communicate findings related to these events with one another. Using the One Health concept of integration and collaboration among health disciplines, the authors systematically review the existing literature to discover where harmful cyanobacteria-associated animal illnesses and deaths have served as sentinel events to warn of potential human health risks. The authors find that illnesses or deaths among livestock, dogs and fish are all potentially useful as sentinel events for the presence of harmful cyanobacteria that may impact human health. The authors also describe ways to enhance the value of reports of cyanobacteria-associated illnesses and deaths in animals to protect human health. Efficient monitoring of environmental and animal health in a One Health collaborative framework can provide vital warnings of cyanobacteria-associated human health risks.

Hollister, J., B. Milstead, and B. Kreakie (2016). Modeling lake trophic state: a random forest approach. *Ecosphere*. ESA Journals, 7(3): e01321.

Productivity of lentic ecosystems has been well studied and it is widely accepted that as nutrient inputs increase, productivity increases and lakes transition from low trophic state (e.g., oligotrophic) to higher trophic states (e.g., eutrophic). These broad trophic state classifications are good predictors of ecosystem health and ecosystem services and disservices (e.g., recreation, aesthetics, fisheries, and harmful algal blooms). While the relationship between nutrients and trophic state provides reliable predictions, it requires *in situ* water quality data in order to parameterize the model. This limits the application of these models to lakes with existing and, more importantly, available water quality data. To expand our ability to predict trophic state in lakes without water quality data, the authors take advantage of the availability of a large national lakes water quality database, land use/land cover data, lake morphometry data, other universally available data, and modern data mining approaches to build and assess models of lake tropic state that may be more universally applied. The authors use random forests and random forest variable selection to identify variables to be used for predicting trophic state and the authors compare the performance of two sets of models of trophic state (as determined by chlorophyll -a concentration). The first set of models estimates trophic state with *in situ* as well as universally available data and the second set of models uses universally available data only. For each of these models the authors used three separate trophic state categories, for a total of six models. It is believed that the presence and abundance of cyanobacteria is strongly associated with trophic state. To test this the authors examined the association between estimates of cyanobacteria abundance and measured chlorophyll-a and find a positive relationship. Expanding these preliminary results to include cyanobacteria taxa indicates that cyanobacteria are significantly more likely to be found in highly eutrophic lakes. These results suggest that predictive models of lake trophic state may be improved with additional information on the landscape surrounding lakes and that those models provide additional information on the presence of potentially harmful cyanobacteria taxa.

Hollister, J., and B. Kreakie (2016). Associations between Chlorophyll-a and various microcystin-LR health advisory concentrations. *F1000 Research*, 5: 151.

Cyanobacteria harmful algal blooms (cHABs) are associated with a wide range of adverse health effects that stem mostly from the presence of cyanotoxins. To help protect against these impacts, several health advisory levels have been set for some toxins. In particular, one of the more common toxins, microcystin, has several advisory levels set for drinking water and recreational use. However, compared to other water quality measures, field measurements of microcystin are not commonly available due to cost and advanced understanding required to interpret results. Addressing these issues will take time and resources. Thus, there is utility in finding indicators of microcystin that are already widely available, can be estimated quickly and in situ, and used as a first defense against high levels of microcystin. Chlorophyll-a is commonly measured, can be estimated in situ, and has been shown to be positively associated with microcystin. In this paper, the authors use this association to provide estimates of chlorophyll-a concentrations that are indicative of a higher probability of exceeding select health advisory concentrations for microcystin-LR. Using the 2007 National Lakes Assessment and a conditional probability approach, the authors identify chlorophyll-a concentrations that are more likely than not to be associated with an exceedance of a microcystin health advisory level. The authors look at the recent EPA health advisories for drinking water as well as the World Health Organization levels for drinking water and recreational use and identify a range of chlorophyll-a thresholds. A 50% chance of exceeding one of the specific advisory microcystin concentrations of 0.3, 1, 1.6, and 2 µg/L is associated with chlorophyll-a concentration thresholds of 24.64, 65.6, 89.71, and 94.93, respectively. When managing for these various microcystin levels, exceeding these reported chlorophyll-a concentrations should be a trigger for further testing and possible management action.

Lin, C., T. Wade, E. Sams , A. Dufour , A. Chapman, and E. Hilborn (2016). A prospective study of marine phytoplankton and reported illness among recreational beachgoers in Puerto Rico, 2009. *Environmental Health Perspectives*, 124(4): 477-483.

The authors evaluated the association between phytoplankton cell counts and subsequent illness among recreational beachgoers. The authors recruited beachgoers at Boquerón Beach, Puerto Rico, during the summer of 2009. The authors conducted interviews at three time points to assess baseline health, water activities, and subsequent illness. Daily water samples were quantitatively assayed for phytoplankton cell count. Logistic regression models, adjusted for age and sex, were used to assess the association between exposure to three categories of phytoplankton concentration and subsequent illness. The authors found associations between recreational exposure to marine phytoplankton and reports of eye irritation, respiratory illness, and rash. The authors also found that associations varied by phytoplankton group, with Cyanobacteria having the strongest and most consistent associations.

Loftin, K., J. Graham, E. Hilborn, S. Lehmann, M. Meyer, J. Dietze, and C. Griffith (2016). Cyanotoxins in Inland Lakes of the United States: Occurrence and Potential Recreational Health Risks in the EPA National Lakes Assessment 2007. *Harmful Algae*. 56:77–90.

A large nation-wide survey of cyanotoxins (1161 lakes) in the United States (U.S.) was conducted during the EPA National Lakes Assessment 2007. Cyanotoxin data were compared with cyanobacteria abundance- and chlorophyll-based World Health Organization (WHO) thresholds and mouse toxicity data to evaluate potential recreational risks. Cylindrospermopsins, microcystins, and saxitoxins were detected (ELISA) in 4.0, 32, and 7.7% of samples with mean concentrations of 0.56, 3.0, and 0.061 µg/L, respectively (detections only). Co-occurrence of the three cyanotoxin classes was rare (0.32%) when at least one toxin was detected. Cyanobacteria were present and dominant in 98 and 76% of samples, respectively. Potential anatoxin-, cylindrospermopsin-, microcystin-, and saxitoxin-producing cyanobacteria occurred in 81, 67, 95, and 79% of samples, respectively. Anatoxin-a and nodularin-R were detected (LC/MS/MS) in 15 and 3.7% samples (n = 27). The WHO moderate and high risk thresholds for microcystins, cyanobacteria abundance, and total chlorophyll were exceeded in 1.1, 27, and 44% of samples, respectively. Complete agreement by all three WHO microcystin metrics occurred in

27% of samples. This suggests that WHO microcystin metrics based on total chlorophyll and cyanobacterial abundance can overestimate microcystin risk when compared to WHO microcystin thresholds. The lack of parity among the WHO thresholds was expected since chlorophyll is common amongst all phytoplankton and not all cyanobacteria produce microcystins.

Mash, H. (2016). Effect of chlorination on the protein phosphatase inhibition activity for several microcystins. *Water Research*, 95: 230-239.

Microcystins are of particular concern due to their toxicity to both humans and animals and are likely the most prominent cyanotoxin observed in freshwater. Although a number of studies have investigated the fate of microcystins and other algal toxins through drinking water treatment facilities, measurement of their potential for toxic activity after chlorination has not been investigated. In this study, six microcystin variants are subjected to chlorine oxidation at several pHs. The removal of six microcystin variants is measured over time. Simultaneously, the measured protein phosphatase inhibition response of the resulting reaction mixture is measured.

Paranjpye, R., W. Nilsson, M. Liermann, E. Hilborn , B. George , Q. Li, B. Bill, V. Trainer, M. Strom, and P. Sandifer (2015). Environmental influences on the seasonal distribution of Vibrio parahaemolyticus in the Pacific Northwest of the USA. *FEMS Microbiology Ecology*. Elsevier Science Ltd, New York, NY, USA, 91(12): 1.

Populations of Vibrio parahaemolyticus in the environment can be influenced by numerous factors. The authors assessed the correlation of total (tl+) and potentially virulent (tdh+) V. parahaemolyticus in water with three harmful algal bloom (HAB) genera (Pseudo-nitzschia, Alexandrium and Dinophysis), the abundance of diatoms and dinoflagellates, chlorophyll-a and temperature, salinity and macronutrients at five sites in Washington State from 2008-2009. The variability in V. parahaemolyticus density was explained predominantly by strong seasonal trends where maximum densities occurred in June, two months prior to the highest seasonal water temperature. In spite of large geographic differences in temperature, salinity and nutrients, there was little evidence of corresponding differences in V. parahaemolyticus density. In addition, there was no evident relationship between V. parahaemolyticus and indices of HAB genera, perhaps due to a lack of significant HAB events during the sampling period. The only nutrient significantly associated with V. parahaemolyticus density after accounting for the seasonal trend was silicate. This negative relationship may be caused by a shift in cell wall structure for some diatom species to a chitinous substrate preferred by V. parahaemolyticus. Results from our study differ from those in other regions corroborating previous findings that environmental factors that trigger vibrio and HAB events may differ depending on geographic locations. Therefore caution should be used when applying results from one region to another.

Sanan, T., N. Dugan , D. Lytle , and H. Mash. Removal of Cyanobacterial Toxins through Drinking Water Treatment on Lake Erie. *Submitted*.

In the summer of 2014 seven drinking water treatment plants (DWTPs) on Lake Erie supplied water samples on a monthly basis for analysis. Chlorophyll-a measurements, LC/MS/MS and ELISA techniques specific to MCs were performed in conjunction with measurements of water quality parameters and nutrients to identify potential harmful algal bloom (HAB) activity. In addition to raw influent waters, samples were collected at varying points through the treatment trains of the DWTPs. Both dissolved and total toxin levels were analyzed, and results showed that microcystin release occurred following oxidant (permanganate) addition in some DWTPs, prior to removal of intact cells. Once the MCs were released, clarification was not efficient in their removal; however, no toxin breakthrough into the distribution system was observed.

Schaeffer, B., K. Loftin, R. Stumpf, and J. Werdell (2015). Agencies Collaborate, Develop a Cyanobacteria Assessment Network. *EOS, Transactions, American Geophysical Union*, 96: 1-9.

This collaborative effort integrates the efforts of the EPA, NASA, NOAA, and the USGS to provide an approach for mainstreaming satellite ocean color capabilities into U.S. fresh and brackish water quality management decisions. The overarching project goal is to support the environmental management and public use of U.S. lakes, reservoirs, and estuaries by providing the capability to detect and quantify cyanobacteria blooms using satellite data records. Satellite remote sensing tools may enable policy makers and environmental managers to assess the sustainability of watershed ecosystems, and the services they provide, under current and future land use practices. Satellite technology allows for the development of cyanobacteria early-warning indicators at the local scale with continuous, synoptic national coverage. The project is structured within a sustainability framework for a more holistic approach including environmental, human health, and economic considerations. The four federal agencies embrace research on sustainability, as recommended by the recent National Research Council report (Sustainability for the Nation: Resource Connection and Governance Linkages), by supporting interdisciplinary research focused on connections among environmental, economic and social problems.

Thompson, J., W. Bartsch, and J. Kelly (2016). Cyanobacteria in nearshore waters across the Great Lakes. In the *National Coastal Condition Assessment 2010*, EPA 841-R-15-006.

This report is based on an analysis of indicators of ecological condition and key stressors in the coastal waters of the Northeast, Southeast, Gulf of Mexico, West, and Great Lakes regions of the conterminous United States. These waters are enormously varied and valuable, including remarkable resources as diverse as Narragansett Bay; the Chesapeake Bay; the subtropical waters of Biscayne Bay and Tampa Bay; San Francisco Bay and Puget Sound; and the nearshore waters of the Great Lakes—the largest expanse of fresh surface water on earth.

Yuan, L.L., and A.I. Pollard (2015) Deriving nutrient targets to prevent excessive cyanobacterial densities in U.S. lakes and reservoirs. Freshwater Biology 60: 1901 – 1916.

High densities of cyanobacteria can interfere with the use of lakes and reservoirs for recreation and as sources for drinking water, and one approach for reducing the amount of cyanobacteria is to reduce nutrient concentrations in the waterbody. An approach is described for deriving numeric targets for concentrations of total phosphorus (TP) and total nitrogen (TN) that are associated with a pre-specified probability of cyanobacterial biovolume that exceeds the recommended World Health Organization thresholds for recreation in the water. A divisive tree algorithm was used to identify groups of lakes in which the relationship between nutrients and cyanobacterial biovolume was similar, followed by hierarchical Bayesian models to estimate relationships between cyanobacterial biovolume, TP and TN, while partitioning the observed variance in biovolume into components associated with sampling variability, temporal variability, and among-lake differences. The final model accounted for 91% of the variance in cyanobacterial biovolume among different lakes and was used to identify nutrient concentrations that maintain a low probability of excessively high cyanobacterial biovolumes. When no classes of lakes were specified and the relationship between cyanobacterial biovolume and nutrient concentrations was modelled using a national data set, mean targets of 87 and 1100 ug/L were derived for TP and TN, respectively, to maintain cyanobacterial biovolume below moderate risk levels as defined by the World Health Organization. After classification, mean nutrient targets in lakes that were found to be most susceptible to high biovolumes of cyanobacteria (i.e., deep lakes) were 61 and 800 ug/ L for TP and TN, while higher nutrient thresholds were observed for other classes of lakes.