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Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin

Draft

Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin

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NOTICES

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FOREWORD

Section 304(a) of the Clean Water Act requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water.

U.S. Environmental Protection Agency (EPA) is publishing these recommended values under Clean Water Act (CWA) 304(a) for states to consider as the basis for swimming advisories for notification purposes in recreational waters to protect the public. Alternatively, states may consider using these same values when adopting new or revised water quality standards (WQS). If adopted as WQS and approved by EPA under CWA 303(c), the WQS could be used for all CWA purposes. States may also wish to consider using these values as both swimming advisory values and WQS. EPA envisions that if states decide to use the values as swimming advisory values they might do so in a manner similar to their current recreational water advisory programs.

This draft document has undergone an EPA intra-agency peer review process. Final review by the Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency has been completed and the document is approved for publication. These values were derived using the existing peer-reviewed and published science on the adverse human health effects of these toxins, established criteria methodologies, and recreation-specific exposure parameters from EPA's Exposure Factors Handbook.

The term “water quality criteria” is used in two sections of the CWA—§304(a)(1) and §303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of effects on human health or aquatic life. The criteria recommendations presented in this document are such a scientific assessment. If water quality criteria associated with specific designated uses are adopted by a state or authorized tribe as water quality standards under section 303, and approved by EPA, they become applicable Clean Water Act water quality standards in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal water quality standards could have the same numerical values as criteria developed under section 304. Alternatively, states and authorized tribes may derive numeric criteria based on other scientifically defensible methods but the criteria must be protective of designated uses. It is not until their adoption as part of state or tribal water quality standards, and subsequent approval by EPA, that criteria become Clean Water Act applicable water quality standards. Guidelines to assist in modifying the criteria recommendations presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 2012b). This handbook and additional guidance on the development of WQS and other water-related programs of this agency have been developed by EPA which along with additional guidance on the development of water quality standards and other water-related programs of this Agency have been developed by the Office of Water.

This document provides recommendations only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

/signed/

Elizabeth Southerland
Director
Office of Science and Technology

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

AWQC	Ambient Water Quality Criteria
BGAS	blue-green algae supplements
bw	body weight
CalEPA	California Environmental Protection Agency
CDC	U.S. Centers for Disease Control and Prevention
CI	confidence interval
CWA	Clean Water Act
CYP450	Cytochrome P450
ELISA	Enzyme Linked Immunosorbent Assays
EPA	U.S. Environmental Protection Agency
GI	gastrointestinal
HAB	harmful algal bloom
HESD	Health Effects Support Document
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
kg	kilograms
K_{oc}	soil organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LOAEL	lowest-observed-adverse-effect-level
LOD	level of detection
LPS	lipopolysaccharide
mL	milliliters
NLA	National Lakes Assessment
NOAEL	no-observed-adverse-effect-level
OATp	organic acid transporter polypeptide
OPP	EPA Office of Pesticide Programs
OR	odds ratio
pg	picogram
RfD	reference dose
ROS	reactive oxygen species
RSC	relative source contribution
SWIMODEL	Swimmers Exposure Assessment Model
TMDL	Total Maximum Daily Load
UF	uncertainty factor
UF_A	uncertainty factor for interspecies variability
UF_D	database uncertainty factor
UF_H	uncertainty factor for intraspecies variability

UF_L	uncertainty factor for LOAEL to NOAEL extrapolation
µg	microgram
USGS	U.S. Geological Survey
WHO	World Health Organization
WQBEL	Water Quality-Based Effluent Limits
WQS	water quality standards

1.0 EXECUTIVE SUMMARY

Cyanobacteria, also commonly referred to as blue-green algae, are photosynthetic bacteria that are ubiquitous in nature, including surface waters. Environmental conditions that promote excessive growth of cyanobacteria in surface waters can lead to situations in which cyanobacterial cell density is high, known as blooms. Environmental factors that play an important role in the development of cyanobacterial blooms and their production of cyanotoxins include the levels of nitrogen and phosphorus, the ratio of nitrogen to phosphorus, temperature, organic matter availability, light attenuation, and pH.

Microcystins can be produced by a variety of cyanobacteria genera including *Microcystis*, *Anabaena*, *Nostoc*, *Oscillatoria*, *Fischerella*, *Planktothrix*, and *Gloeotrichia*. Some of these species can be distributed through the water column, concentrate in the upper layers, or form surface scums depending on environmental conditions. More than 100 microcystin congeners exist, which vary based on amino acid composition. The majority of toxicological data on the effects of microcystins are available for microcystin-LR, which is also a frequently monitored congener. Microcystins are water-soluble and tend to remain contained within the cyanobacterial cell, until the cell breaks, and they are released into the water. Microcystins typically have a half-life of 4 to 14 days in surface waters or may persist longer, depending on such factors as the degree of natural degradation owing to sunlight, organic matter, and the presence of bacteria. Microcystins can persist even after a cyanobacterial bloom is no longer visible.

Cylindrospermopsin can be produced by a variety of cyanobacteria species including *Cylindrospermopsis raciborskii*, *Aphanizomenon* species, *Anabaena* species, *Lyngbya wollei*, and *Rhaphidiopsis* species. Some of these species tend not to form visible surface scums, and the highest concentrations of cyanobacterial cells typically occur below the water surface. Cylindrospermopsin may be retained within the cell or released into the water. The biodegradation of cylindrospermopsin in natural water bodies is a complex process that can be influenced by many environmental factors, including toxin concentration, water temperature, sunlight, and the presence of cell pigments and bacteria. Half-lives of 11 to 15 days and up to 8 weeks have been reported for cylindrospermopsin in surface waters.

This document for microcystins and cylindrospermopsin focuses on the human health risks associated with recreational exposures in waters containing these cyanotoxins. Exposure to cyanobacteria and their toxins can also occur through non-recreational pathways such as consumption of cyanotoxin-contaminated drinking water and food (including fish), and during bathing or showering. The non-recreational exposures were not quantified in the recreational exposure scenario described herein. Given that cyanobacterial blooms typically are seasonal events, recreational exposures are likely to be episodic, and may be short-term in nature.

U.S. Environmental Protection Agency (EPA) is publishing these recommended values for microcystins and cylindrospermopsin under Clean Water Act (CWA) 304(a) for states to consider as the basis for swimming advisories for notification purposes in recreational waters to protect the public. Additionally, states may consider using these same values when adopting new or revised water quality standards (WQS). If adopted as WQS and approved by EPA under CWA 303(c), the WQS could be used for all CWA purposes. States may also wish to consider using these values as both swimming advisory values and/or WQS. EPA envisions that if states decide to use the values as swimming advisory values they would do so in a manner similar to their

current recreational water advisory programs. The recommended values for use as swimming advisories and/or WQS leverage the information collected and evaluated in EPA's *Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins* and *Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin* (Drinking Water Health Advisories) for these cyanotoxins.

At this time, available data are insufficient to develop quantitative recreational values for cyanobacterial cell density related to inflammatory health endpoints. The reported epidemiological relationships in the literature are not consistent for specific health outcomes (e.g., dermal symptoms, eye/ear irritation, fever, gastrointestinal (GI) illness, and respiratory symptoms) or for those health outcomes associated with specific cyanobacterial cell densities. The uncertainties related to the epidemiological study differences, such as study size, species and strains of cyanobacteria present, and the cyanobacterial cell densities associated with significant health effects, do not provide sufficient information to determine a consistent association between cyanobacterial densities associated with adverse inflammatory health effects.

EPA evaluated the health effects of microcystins and derived a Reference Dose (RfD) in its 2015 *Health Effects Support Document for the Cyanobacterial Toxin Microcystins.* Exposure to higher-levels of microcystins can lead to liver damage and renal failure. The critical study for the derivation of the microcystins RfD was conducted by Heinze et al. (1999) based on rat exposure to microcystin-LR in drinking water. The critical effect from this study was liver damage, including increased liver weight, slight to moderate liver lesions with hemorrhages, and increased liver enzyme levels. EPA established an RfD for microcystin-LR and used it as a surrogate for other microcystin congeners.

EPA evaluated the health effects of cylindrospermopsin and derived an RfD in its 2015 *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin.* The kidneys and liver appear to be the primary target organs for cylindrospermopsin toxicity. The critical study for the derivation of the cylindrospermopsin RfD was conducted by Humpage and Falconer (2002, 2003) based on drinking water exposure to mice. The critical effect was kidney damage, including increased kidney weight and decreased urinary protein.

Based on available noncancer health effects information, EPA is recommending values protective of primary contact recreation for two cyanotoxins as follows:

- For microcystins, the recreational value is 4 micrograms (µg)/liter (L).
- For cylindrospermopsin, the recreational value is 8 µg/L.

These values are based on overall exposure to children at the 90th percentile. If used as a swimming advisory to protect swimmers at a beach, these values are not to be exceeded on any single day. If used as a water quality criterion for assessment and listing purposes, EPA recommends that states consider the number of exceedances of no more than 10 percent of days per recreational season up to one year. These criteria are based on noncancer health effects because EPA concluded in its Health Effects Support Documents (HESDs) for microcystins and cylindrospermopsin that there is inadequate information to assess carcinogenic potential of these cyanotoxins (U.S. EPA 2015c; U.S. EPA 2015d). Should additional information become available in the future, EPA can review and revise these recommendations, as appropriate.

2.0 INTRODUCTION AND BACKGROUND

This section provides background information about cyanobacteria and cyanobacterial blooms, the source of the stressors, microcystins and cylindrospermopsin. It discusses briefly the occurrence of cyanobacterial blooms and these cyanotoxins in the United States. Section 2.1 describes Clean Water Act provisions relevant to these recreational ambient water quality criteria or swimming advisories. Section 2.2 summarizes international and state recreational water guidelines for microcystins, cylindrospermopsin, and cyanobacteria to provide context regarding how other jurisdictions are addressing the human health concerns.

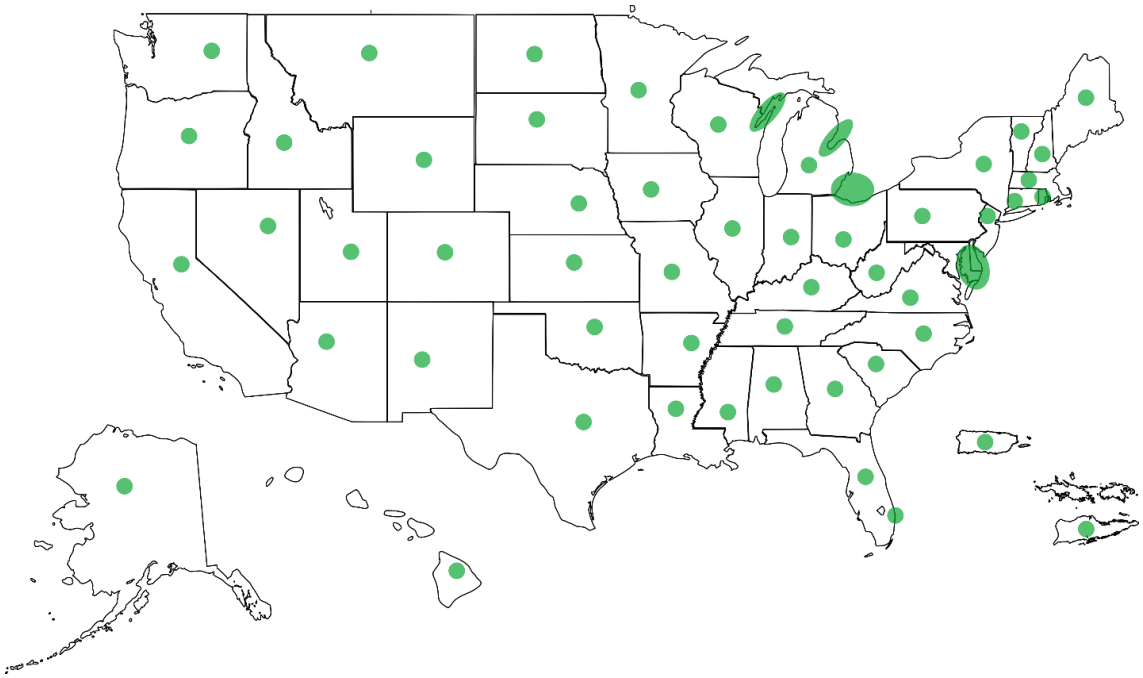
Cyanobacteria are a group of microorganisms that naturally occur in freshwater and marine environments and can be found at higher densities in eutrophic or nutrient-enriched water bodies. Many cyanobacteria are capable of producing toxins, generally referred to as cyanotoxins, which can impact human health. Under the right conditions of temperature, light, pH, nutrient availability, etc., cyanobacteria can reproduce rapidly to high densities in water, forming what are commonly referred to as cyanobacterial harmful algal blooms (HABs). Other microorganisms can form HABs, but for the purpose of this document, which addresses cyanotoxins, usage of "HABs" will be in reference to cyanobacterial HABs unless otherwise specified. A variety of factors can influence both cyanobacteria proliferation and toxin production, including nutrient (e.g., nitrogen and phosphorus) concentrations, light levels, temperature, pH, oxidative stressors, and interactions with other biota (viruses, bacteria, and animal grazers), and others, as well as their combined effects (Paerl & Otten 2013a; Paerl & Otten 2013b).

Because they are a natural part of algal communities, cyanobacteria are commonly observed in freshwater systems. The occurrence of HABs has been documented in surface waters of all 50 states as well as U.S. territories between 2006 and 2015 as shown in Figure 2-1 (Richlen 2016; WHOI 2016). Figure 2-1 also identifies areas where more widespread HAB problems have occurred, e.g., parts of the Great Lakes.

In 2007, the EPA's National Lakes Assessment (NLA) conducted a national probability-based survey of the nation's lakes, ponds, and reservoirs (Loftin et al. 2016b; U.S. EPA 2009). These surveys covered a total of 1,028 lakes, which represented nearly 50,000 lakes larger than 4 hectares (10 acres) in the conterminous United States. This assessment found that cyanobacteria were detected in almost all lakes (U.S. EPA 2009). Cyanobacteria were the dominant member of the phytoplankton community in 76 percent of lake samples. Subsequent analysis indicated that potential microcystin- and cylindrospermopsin- producing species occurred in 95 and 67 percent of samples, respectively (Loftin et al. 2016b).

Microcystins are the most commonly detected class of cyanotoxin and have been found in lakes in the contiguous United States (U.S. EPA 2009) and streams in the Southeastern United States (Loftin et al. 2016a). The NLA 2007 reported that 30 percent of lakes in the conterminous United States had detectable microcystin. In a separate study, Graham et al. (2010) sampled cyanobacterial blooms in 23 Midwestern lakes and detected microcystins in all blooms sampled. The researchers also found that cylindrospermopsin co-occurred with microcystins in 9 percent of samples (Graham et al. 2010). In an expanded analysis of NLA samples, Loftin et al. (2016b) identified cylindrospermopsin in 4 percent of samples with a mean concentration of 0.56 µg/L.

Figure 2-1. Generalized Distribution of Cyanobacterial HABs in the United States and Territories[a]



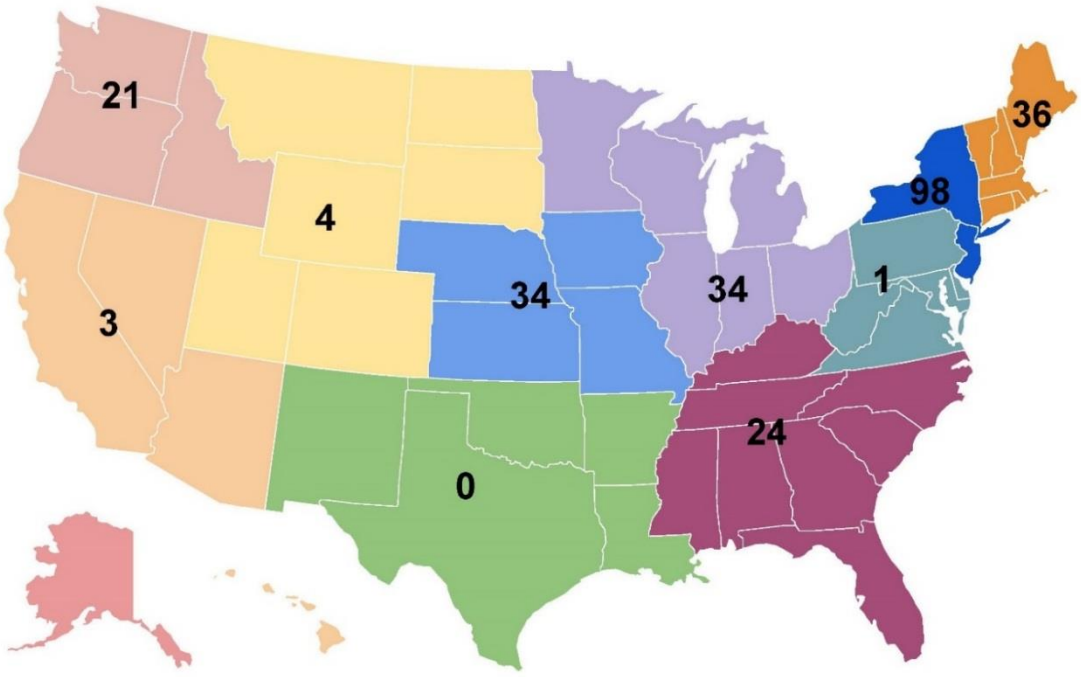
[a] Graphic adapted from a Woods Hole Oceanographic Institute (WHOI) map of HABs that occurred between 2006 and 2015. It reflects input from HAB experts with broad experience in HAB events and reports to the U.S. National Office for Harmful Algal Blooms (Richlen 2016; WHOI 2016). Each state that has experienced one or more cyanobacterial HABs is indicated with a single green dot. Larger green ovals mark areas where more widespread cyanobacterial HAB problems occurred.

Exposure to HABs can result in adverse human health outcomes such as gastrointestinal, dermatologic, respiratory, neurologic, and other symptoms. The Centers for Disease Control and Prevention (CDC) collected information on outbreaks of illness related to HABs reported via the National Outbreak Reporting System (NORS) and the Harmful Algal Bloom-Related Illness Surveillance System (HABISS). During 2009 and 2010 in the United States, 11 waterborne disease outbreaks associated with HABs were reported to CDC, all occurring in freshwater lakes: eight of these investigations evaluated the presence of cyanotoxins; eight detected microcystins; and two detected cylindrospermopsin (Hilborn et al. 2014). The 11 outbreaks associated with HABs affected at least 61 persons, resulting in 2 hospitalizations; 66 percent of the affected persons overall were aged 18 or younger, and 35 percent were aged 9 years or younger. Hilborn et al. (2014) reported that microcystins were present during all eight outbreak investigations in which cyanotoxin testing was performed. In four of the outbreaks, microcystin concentrations exceeded 20 µg/L. During investigations of these outbreaks, cylindrospermopsin, and anatoxin-a also were detected. The researchers concluded that the disease outbreak data suggest that the time to onset of effects might be rapid, that children might be at higher risk for illness, and that HAB–associated outbreaks occur during the warmer months. Hilborn et al. (2014) noted that recognizing HAB-associated illness from recreational exposure might be underreported due to multiple possible exposure routes and the non-specific nature of potential health effects. In addition, Graham et al. (2009) counted 36 states with anecdotal reports of acute cyanotoxin poisonings of animals, humans, or both as reported in journal articles and newspaper articles (Chorus & Bartram 1999; Huisman et al. 2005; Yoo et al. 1995).

As a result of potential adverse health effects associated with recreational exposure to HABs, many states have developed guidelines and/or health advisories related to HABs. For the summer of 2008, Graham et al. (2009) identified at least 13 states that posted recreational health advisories because the concentrations of cyanobacteria or cyanotoxins were large enough to be considered a risk to animals and people by the state (Graham et al. 2009). These included cautions, warnings, public health advisories, and public health warnings, due to the presence of cyanobacteria, cyanotoxins, or both.

Figure 2-2 shows the number of 2016 freshwater HAB recreational advisories states publicly reported in each EPA region between January 1 and August 12, 2016. To develop this regional summary map, EPA researched and compiled publically available reports posted on states' websites between these dates. During that time, states reported at least 255 notices for freshwater HABs with reported microcystins concentrations ranging from not detected (i.e., below the limit of detection) to 392 µg/L. These notices included cautions, warnings, public health advisories, and public health warnings due to the presence of cyanobacteria, cyanotoxins, or both. Advisories can last for multiple days. The review was not exhaustive and might not reflect all of the monitoring, beach, or general health advisories (e.g., some advisories at local or county-level may not be posted on the state website). Thus, the number of actual HAB advisories during this time might be higher. In addition, many states have only recently begun to monitor HABs, thus monitoring may be limited.

Figure 2-2. State-reported HAB Advisories by EPA Region, January 1 to August 12, 2016



The presence of detectable concentrations of cyanotoxins in the environment is closely associated with HAB occurrences. Cyanotoxin concentrations in surface waters can be higher after the initial bloom fades, so potential exists for human exposures even after the visible signs of a bloom are gone. Thus, high densities of cyanobacteria and high cyanotoxin concentrations are capable of affecting the health of humans, domestic animals, and wildlife in contact with

affected waters. These events are not always independent; animal health effects associated with harmful cyanobacteria have served as sentinel events to warn of potential human health risks (Hilborn & Beasley 2015). Cyanotoxin production by cyanobacteria can vary spatially and temporally, and studies of the impacts of environmental factors on cyanotoxin production are ongoing.

Nutrients are key environmental drivers that influence the proportion of cyanobacteria in the phytoplankton community, the cyanobacterial biovolume, cyanotoxin production, and the impact that cyanobacteria may have on ecosystem function and water quality (Paerl et al. 2011). Cyanobacteria production and cyanotoxin concentrations are dependent on nutrient levels (Wang et al. 2002); however, nutrient uptake rates and the utilization of organic and inorganic nutrient forms of nitrogen and phosphorus vary considerably by cyanobacteria species. In addition to nutrient concentrations, factors such as the nitrogen:phosphorus ratio and organic matter availability, as well as other physico-chemical processes, can play a role in determining HAB composition and cyanotoxin production (Paerl & Huisman 2008; Paerl & Otten 2013b).

2.1 Clean Water Act

Section 304(a) of the Clean Water Act (CWA) requires the Administrator of EPA to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water.

EPA is publishing these recommended values under CWA 304(a) for states to consider as the basis for swimming advisories for notification purposes in recreational waters to protect the public. Additionally, states may consider using these same values as criteria when adopting new or revised WQS. If adopted as WQS and approved by EPA under CWA 303(c), the WQS could be used for CWA purposes. States may also wish to consider using these values as both swimming advisory values and WQS. EPA envisions that if states decide to use the values as swimming advisory values they might do so in a manner similar to their current recreational water advisory programs.

This document recommends values for cyanotoxins that are protective of human health given a primary contact recreational exposure scenario. The cyanotoxins included in this document have been demonstrated to occur in nutrient-enriched waters affected by cyanobacterial HABs.

The term “water quality criteria” is used in two sections of the CWA§304(a)(1) and §303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of effects on human health or aquatic life. The criteria recommendations presented in this document are such a scientific assessment. If water quality criteria associated with specific designated uses are adopted by a state or authorized tribe as water quality standards under section 303, and approved by EPA, they become applicable CWA water quality standards in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal water quality standards could have the same numerical values as criteria developed under section 304. Alternatively, states and authorized tribes may derive numeric criteria based on other scientifically defensible methods, but the criteria must be protective of designated uses. It is not until their adoption as part of state or tribal water quality standards, and subsequent approval by EPA, that criteria become CWA applicable water quality standards.

Guidelines to assist in modifying the criteria recommendations presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 2012b). This handbook and additional guidance on the development of WQS and other water-related programs of this Agency have been developed by EPA, which along with additional guidance on the development of water quality standards and other water-related programs of this Agency have been developed by the Office of Water.

2.2 International and State Guidelines

In 2003, World Health Organization (WHO) derived a series of guideline values for recreational exposure to cyanobacteria associated with incremental severity and probability of adverse health effects (WHO 2003b); see Table 2-1. For these guidelines, WHO recommended values that included the potential health effects from exposure to cyanobacteria because it was "unclear whether all important cyanotoxins had been identified and that the health outcomes observed after recreational exposure could be related to cyanobacterial substances other than the well-known cyanotoxins (WHO 2003b)." They also considered the potential for liver damage by microcystins. WHO highlighted that there are multiple potential health endpoints related to recreational exposure to cyanobacteria and their toxins and developed a series of guidelines associated with incremental severity and probability of health effects at increasing densities of cyanobacteria and corresponding concentrations of chlorophyll *a* (if cyanobacteria dominate). The different levels were an effort to distinguish between irritative or inflammatory-response symptoms associated with cyanobacterial cells and the more severe hazard of exposure to elevated concentrations of cyanotoxins, particularly microcystins. The WHO guidelines combine the potential for both sets of endpoints (i.e., cyanotoxins and cyanobacterial cells) across three categories of increasing probability of risk. The cell-associated inflammatory responses are represented by the low probability of adverse health effects category of < 20,000 cells/milliliter (mL), corresponding to less than 10 µg/L chlorophyll *a* if cyanobacteria dominate and estimated microcystin levels of less than 10 µg/L. According to the WHO, as the density of cyanobacteria increase above that level, the probability of inflammatory responses increases, and the potential for more severe adverse health effects associated with exposure to the cyanotoxins also increases. The high-risk category identified by WHO related > 100,000 cells/mL (corresponding to 50 µg/L of chlorophyll *a*, if cyanobacteria dominate) and > 20 µg/L microcystin levels, was primarily due to the toxic effects of microcystins.

Table 2-1. WHO (2003b) Recreational Guidance/Action Levels for Cyanobacteria, Chlorophyll *a,* and Microcystin

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Chlorophyll *a* (µg/L)	Estimated Microcystin Levels (µg/L)[a]
Low	< 20,000	< 10	< 10
Moderate	20,000–100,000	10–50	10–20
High	>100,000–10,000,000	50–5,000	20–2,000
Very High	> 10,000,000	> 5,000	> 2,000

[a] WHO (2003b) derived the microcystin concentrations from the cyanobacterial cell density levels.

The WHO guideline value development was supported by results from a review conducted by Chorus and Bartram (1999). A primary study identified in this review was a prospective epidemiological study by Pilotto et al. (1997), which evaluated health effects after recreational exposure to cyanobacteria and reported associations between cyanobacterial cell densities and health. Pilotto et al. (1997) found a significant association among recreators exposed to > 5,000 cells/mL for > 1 hour and one or more symptoms and similar significant associations for symptoms in people exposed to 5,000–20,000 cells/mL. WHO chose a guideline level of 20,000 cells/mL to represent the upper bound of the low probability of adverse health effects category (WHO 2003b). The low category includes irritative or inflammatory health effects associated with exposure to cyanobacterial cells (WHO 2003b). While the association among recreators exposed to > 5,000 cells/mL for > 1 hour and one or more symptoms reported in Pilotto et al. (1997) were statistically significant, WHO states that they represented less than 30 percent of the individuals exposed (Chorus & Bartram 1999). Therefore, the level of health effect and the small number of people affected at 5,000 cells/mL were not considered by WHO to be a basis to justify action (WHO 2003b).

WHO (2003b) also made the connection between cyanobacterial cell densities and microcystin concentrations. It assumed microcystin-producing cyanobacteria dominate the population of cyanobacteria present and that the average microcystin content of *Microcystis* sp. cells averages 0.2 pg/cell. Thus, WHO estimated that 20,000 cells/mL could potentially equate to approximately 2-4 μg/L of microcystin. Similarly, using the same assumptions at a cyanobacterial cell density of 100,000 cells/mL, they estimated approximately 20 μg/L. WHO pointed out that the potential concentration of microcystins could vary based on the composition of the community of cyanobacteria present. WHO states that, at the same cyanobacterial cell density, cyanotoxin levels may approximately double if *Planktothrix agardhii* is the dominant member of the community. Several states have adopted the estimated microcystins concentrations as their guideline values rather than the cell density or chlorophyll *a* values from the WHO guidelines, as discussed later in this section.

Many countries have adopted the WHO recommendations for recreational waters including multiple parameters (e.g., cell density, biovolume, and cyanotoxin concentration). Table 2-2 provides international recreational water guideline or action levels for cyanotoxins or cyanobacteria for several countries. Table 2-2 lists the lowest concentrations of cyanotoxins or densities of cyanobacteria that prompt a health protective action. For a more complete list of guideline or action levels and recommended actions for international jurisdictions, see Appendix A. EPA did not identify any recreational guideline levels for cylindrospermopsin established by other international regulatory authorities. Some international authorities have multiple action levels; for brevity, Table 2-2 that follows presents the guideline reflecting *the lowest concentration* of microcystin or density of cyanobacterial cells that recommended or triggered a health protective action. More details are in Appendix A.

Table 2-2. International Recreational Water Guideline or Action Levels for Cyanobacteria and Microcystins

Jurisdiction	Lowest Recreational Water Guideline/Action Level[a]	Reference
Australia[b]	microcystins (total): ≥ 10 µg/L or *Microcystis aeruginosa* (total): ≥ 500 to < 5,000 cells/mL or cyanobacteria (total): ≥ 0.4 to < 4 mm^3/L (where a known toxin producer is dominant in the total biovolume)	Australian Government National Health and Medical Research Council (2008)
Canada	microcystins (total): ≥ 20 µg/L (expressed as microcystin-LR) or cyanobacteria (total): ≥ 100,000 cells/mL	Health Canada (2012)
Cuba	cyanobacteria: > 1 of the species known as potentially toxic or phytoplankton cells: > 20,000 – to < 100,000 cells/mL, > 50 percent of cells cyanobacteria	German Federal Environment Agency (2012)[c]
Czech Republic	cells: >20,000 cells/mL	German Federal Environment Agency (2012)[c]
Denmark	chlorophyll *a*: >50 µg/L, dominated by cyanobacteria or visible surface scum	German Federal Environment Agency (2012)[c]
European Union	Appropriate monitoring must be implemented if there is a risk of proliferation of algae. Member state authorities responsible must take management measures and provide information immediately if a proliferation of cyanobacteria (or blue algae) occurs.	European Parliament and the Council of the European Union (2006)
Finland	algae (includes cyanobacteria): detected	German Federal Environment Agency (2012)[c]
France[b]	microcystins: > 25 µg/L or cyanobacteria: > 20,000 to < 100,000 cells/mL (±20 percent)	German Federal Environment Agency (2012)[c]
Germany	Secchi Disk reading > 1 m AND (microcystins: ≥ 10 µg/L or chlorophyll *a* (with dominance by cyanobacteria): ≥ 40 µg/L or biovolume: ≥ $1mm^3/L$)	German Federal Environment Agency (2012)[c]
Hungary	microcystins: ≥ 4 to < 10 µg/L or cell count: ≥ 20,000 to < 50,000 cells/mL or chlorophyll *a* (with dominance by cyanobacteria): ≥ 10 to < 25 µg/L	German Federal Environment Agency (2012)[c]
Italy[b]	microcystins: > 25 µg/L or cyanobacterial cell count (combined with identification of genus and, if possible, species): > 20,000 cells/mL	German Federal Environment Agency (2012)[c]
Netherlands	chlorophyll *a*: ≥12.5 to ≤ 75 µg/L or biovolume (cyanobacterial cell count): ≥ 2.5 to ≤ 15 mm^3/L	German Federal Environment Agency (2012)[c]
New Zealand[b]	microcystins (total): ≥ 12 µg/L or cyanobacteria (benthic): 20−50 percent coverage of potentially toxigenic cyanobacteria attached to substrate or cyanobacteria (total): > 0.5 to < 1.8 mm^3/L (biovolume equivalent of potentially toxic cyanobacteria) or cyanobacteria (total): > 0.5 to < 10 mm^3/L (biovolume equivalent of the combined total of all cyanobacteria)	Wood et al. (2008)

Jurisdiction	Lowest Recreational Water Guideline/Action Level[a]	Reference
Poland	visible blooms	German Federal Environment Agency (2012)[c]
Scotland[b]	chlorophyll *a*: ≥ 10 µg/L with dominance of cyanobacteria or cyanobacteria: ≥ 20,000 cells/mL	Scottish Government Health and Social Care Directorates Blue-Green Algae Working Group (2012)
Spain	cyanobacteria proliferation potential (Low)	German Federal Environment Agency (2012)[c]
Turkey	microcystin-LR: > 25 µg/L equivalents or cells: ≥ 20,000 to 100,000 cells/mL	German Federal Environment Agency (2012)[c]
World Health Organization (WHO)	cyanobacteria: 20,000 cells/mL or chlorophyll *a*: 10 µg/L (approximately 2–4 µg microcystin/L, assuming cyanobacteria dominance)	Chorus and Bartram (1999); WHO (2003b)

[a] More details are provided in Appendix A.

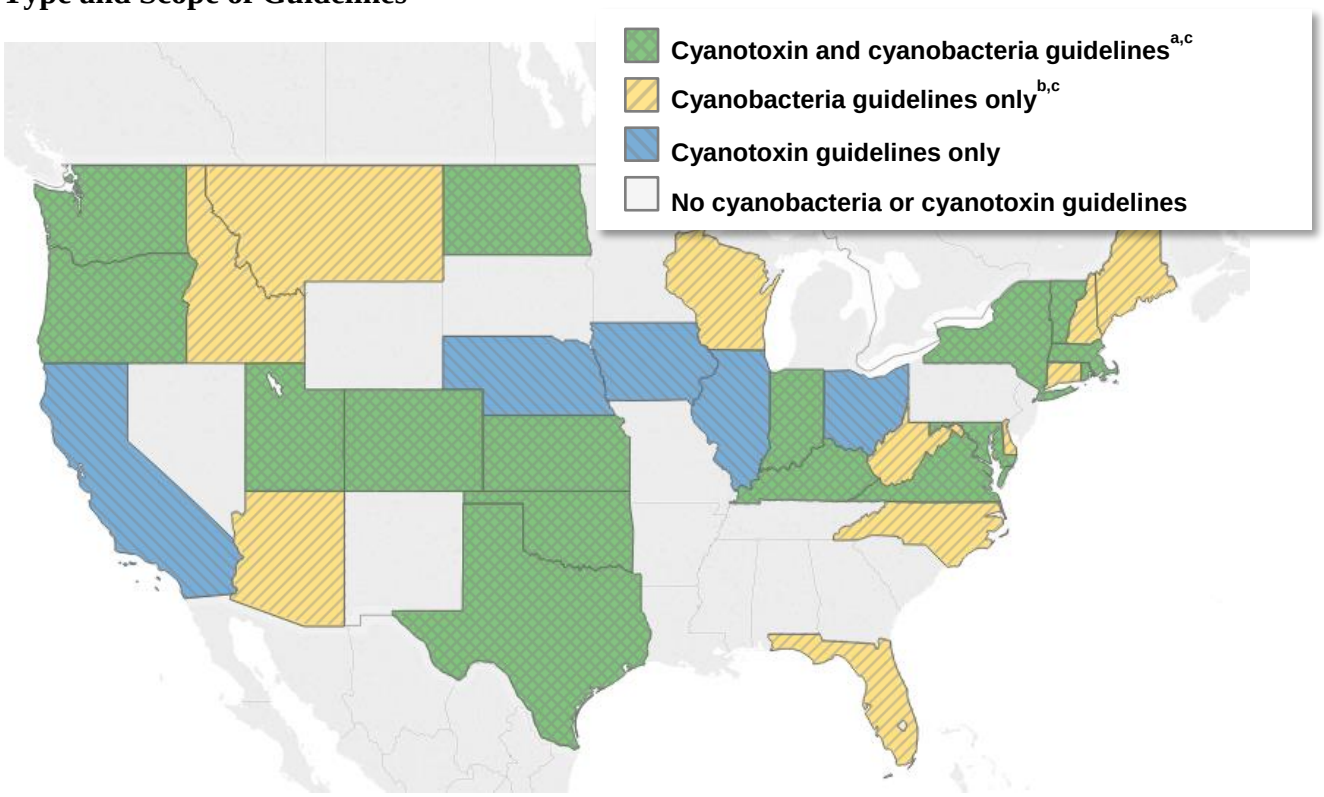
[b] The lowest guideline values for each quantitative parameter (i.e., cyanobacterial cell density, biovolume, cyanotoxin concentration) are not associated with the same action level. For example, for Australia, the lowest cyanobacterial cell density and biovolume criteria trigger the green level surveillance mode, and the lowest cyanotoxin concentration triggered the red level action mode.

[c] Following the VIIIth International Conference on Toxic Cyanobacteria, the German Federal Environmental Agency compiled and published in 2012 regulatory approaches to the assessment and management of cyanotoxin risks based on contributions by member countries.

Approximately 30 U.S. states have implemented cyanobacterial HAB guidelines for recreational waterways as of November 2015. As shown in Figure 2-3, five states had quantitative cyanotoxin guidelines only, and fourteen states had quantitative guidelines for cyanotoxins, as well as either quantitative or qualitative guidelines for cyanobacterial cell density. Generally, qualitative guidelines use visual inspection and not quantitative detection methods. In addition, twelve states had quantitative guidelines for cyanobacterial cell density only or had qualitative guideline values only.

For brevity, Table 2-3 lists the lowest recreational water guideline or action levels for microcystins, cylindrospermopsin, or cyanobacteria that trigger or recommend a health protective action for U.S. states. For a more complete list of state guideline/action levels and recommended actions see Appendix B. Parameters and values used as the basis for guidelines varied across states, as does the methodology for developing the values. Similar to international authorities, many states used a tiered approach, which evaluates multiple parameters including cyanobacterial cell density, chlorophyll *a* concentration, cyanotoxin concentration, and visual appearance. New York, for example, considered all four of these parameters at lower tier guideline levels, but only considered cyanotoxin concentrations at the highest advisory level. Other states had only one response guideline level and only consider cyanotoxin concentration (e.g., California) or had only one response guideline level, but considered cyanobacterial cell density, cyanotoxin concentration, and visual appearance (e.g., Oregon). In contrast, other states, like Connecticut, used a tiered approach and did not consider cyanotoxin concentrations at any tier but rather consider visual inspection and cyanobacterial cell density.

Figure 2-3. State Guidelines for Cyanotoxins and Cyanobacteria in Recreational Water by Type and Scope of Guidelines



[a] Includes states with quantitative cyanotoxin guidelines as well as either quantitative or qualitative cyanobacteria guidelines.

[b] Includes states that either have quantitative cyanobacteria guidelines only or qualitative guidelines only.

[c] EPA found that Texas and North Carolina published guidelines in the past, but the guidelines were no longer on their websites.

Table 2-3. State Guideline or Action Levels for Microcystin, Cylindrospermopsin, and Cyanobacterial Cells in Recreational Water

State	Lowest Recreational Water Guideline or Action Level[a]	Reference
Arizona	blue-green algae (mean value based on a minimum of two sample events within one peak season): 20,000 cells/mL and chlorophyll *a* result (mean value based on a minimum of two sample events within one peak season) in target range	Arizona Department of Environmental Quality (2008)
California	microcystins: 0.8 µg/L	Butler et al. (2012)
	cylindrospermopsin: 4 µg/L	
Colorado	microcystin-LR: ≥ 10 µg/L and < 20 µg/L	Colorado Department of Public Health & Environment (2016)
	cylindrospermopsin: ≥ 7 µg/L	
Connecticut	visual rank category 2: cyanobacteria present in low numbers; there are visible small accumulations but	Connecticut Department of Public Health: Connecticut Energy

State	Lowest Recreational Water Guideline or Action Level[a]	Reference
	water is generally clear; OR blue-green algae cells > 20,000 cells/mL and < 100,000 cells/mL	Environment (2013)
Delaware	thick green, white, or red scum on surface of pond	Delaware Department of Natural Resources and Environmental Control: Division of Water (2016)
Florida	cyanobacteria bloom	Florida Department of Environmental Protection (2016); Florida Department of Health (2016)
Idaho	*Microcystis* or *Planktothrix*: > 40,000 cells/mL	IDEQ (2015)
	sum of all potentially toxigenic taxa: ≥ 100,000 cells/mL	
Illinois	microcystin-LR: > 10 µg/L	Illinois Environmental Protection Agency (2015)
Indiana	blue-green algae: 100,000 cells/mL	Indiana Department of Environmental Management (2015)
	microcystin-LR: 6 µg/L	
	cylindrospermopsin: 5 µg/L	
Iowa	microcystin: ≥ 20 µg/L	Iowa Environmental Council (2015)
Kansas	cyanobacteria: ≥ 80,000 and < 250,000 cells/mL	Kansas Department of Health & Environment (2015)
	microcystin: ≥ 4 and < 20 µg/L	
Kentucky	blue-green algae: > 100,000 cells/mL	Kentucky Department for Environmental Protection (2014)
	microcystins: > 20 µg/L	Commonwealth of Kentucky: Department for Environmental Protection Division of Water (2015)
Maine	Secchi disk reading < 2 meters caused by algae	Maine Department of Environmental Protection (2013)
Maryland	*Microcystis aeruginosa* or other potential microcystin producing blue green algae > 40,000 cells/mL, and samples contain microcystins: > 10 ppb	Maryland Department of Natural Resources (2010)
Massachusetts	blue-green algae: > 50,000 cells/mL	Massachusetts Bureau of Environmental Health (2015)
	microcystins: > 14 µg/L	
Montana	reservoirs that seem stagnated and harbor large quantities of algae	State of Montana Newsroom (2015)
Nebraska	microcystin: ≥ 20 µg/L	Nebraska Department of Environmental Quality and Nebraska Department of Health and Human Services: Division of Public Health (2016)
New Hampshire	cyanobacteria: > 50 percent of total cell counts from toxigenic cyanobacteria	New Hampshire Department of Environmental Services (2014)

State	Lowest Recreational Water Guideline or Action Level[a]	Reference
New York	bloom: credible report or digital imagery of a bloom determined as likely to be potentially toxic cyanobacteria by DEC or DOH staff	Gorney (2016)
	blue green chlorophyll *a*: > 25–30 µg/L	
	potential toxin-producing cyanobacteria taxa: > 50 percent of algae present	
	microcystin-LR: 4 µg/L	
North Carolina	visible discoloration or surface scum	North Carolina Health and Human Services: Division of Public Health (2014)
North Dakota	blue-green algae bloom is present over a significant portion of the lake AND microcystin-LR: ≥ 10 µg/L	North Dakota Department of Health: Division of Water Quality (2016)
Ohio	microcystin-LR: 6 µg/L	Kasich et al. (2015)
	cylindrospermopsin: 5 µg/L	
Oklahoma	cyanobacteria: 100,000 cell/mL	Oklahoma Legislature (2012)
	microcystin: > 20 µg/L	
Oregon	cylindrospermopsin: ≥ 20 µg/L	Oregon Health Authority (2016)
	microcystin: ≥ 10 µg/L	
	Microcystis: > 40,000 cells/mL	
	Planktothrix: > 40,000 cells/mL	
	toxigenic species: > 100,000 cells/mL	
	visible scum with documentation and testing	
Rhode Island	cyanobacteria: > 70,000 cells/mL	Rhode Island Department of Environmental Management and Rhode Island Department of Health (2013)
	microcystin-LR: ≥ 14 µg/L	
	visible cyanobacteria scum or mat	
Texas	> 100,000 cell/mL of cyanobacteria cell counts and > 20µg/L microcystin	U.S. EPA (2016)
Utah	blue-green algae: 20,000–100,000 cells/mL	Utah Department of Environmental Quality and Department of Health (2015)
	microcystin: 4–20 µg/L	

State	Lowest Recreational Water Guideline or Action Level[a]	Reference
Vermont	cylindrospermopsin: ≥ 10 µg/L	Vermont Department of Health (2015)
	microcystin-LR (equivalents): ≥ 6 µg/L	
	visible known blue-green algae bloom/scum or an unknown, potentially blue-green algae (i.e., not pollen), bloom/scum	
Virginia	blue-green algal "scum" or "mats" on water surface	Virginia Department of Health (2012)
	microcystin: > 6 µg/L	
	Microcystis: 5,000 to < 20,000 cells/mL	
Washington	bloom is forming or a bloom scum is visible (toxic algae may be present); cyanotoxin levels do not exceed thresholds	Hardy and Washington State Department of Health (2011)
	microcystins: 6 µg/L	
	cylindrospermopsin: 4.5 µg/L	
West Virginia	blue-green algal blooms observed and monitored	West Virginia Department of Health & Human Resources (2015)
Wisconsin	cyanobacteria: > 100,000 cells/mL	Wisconsin Department of Natural Resources (2012)
	visible scum layer	Werner and Masnado (2014)

[a] More details are provided in Appendix B.

Among states that consider the same parameters, there is considerable variation in guideline levels and associated responses. As shown in Table 2-3, the state recreational guidelines featuring the lowest microcystins or cylindrospermopsin concentrations that recommended or triggered a health protective action ranged from 0.8 to 20 µg/L and 4 to 20 µg/L, respectively. The guidelines reflecting the lowest densities of cyanobacterial cells that triggered a health protective action ranged from 5,000 to 100,000 cells/mL. Some of the variation in guideline levels is attributable to variations in exposure parameters, as well as variations in the basis for guideline values. Ten states base at least one guideline value on either WHO guidance or a modified version of WHO guidance (e.g., Indiana, Oregon, Utah). Eleven states, including California, Massachusetts, and Ohio, base at least one guideline value on jurisdiction-specific risk assessments or monitoring information, or studies or guidelines other than those from WHO. For more information on individual state guidelines, see Appendix B.

3.0 NATURE OF THE STRESSORS

This section discusses cyanobacteria and cyanobacterial blooms that have the potential to produce microcystins and cylindrospermopsin. It also describes these toxins' chemical and physical properties, sources and occurrence information in different media, environmental fate, and toxicokinetics.

3.1 Cyanobacteria and Cyanobacterial Blooms

Cyanobacteria are photosynthetic prokaryotes (Seckbach & Oren 2007) and are ubiquitous in the environment. The chloroplast, found in photosynthetic eukaryotes like algae and plants, evolved from an endosymbiotic relationship with cyanobacteria (Kutschera & Niklas 2005). Ecologists historically grouped cyanobacteria, often referred to as "blue-green algae," with eukaryotic algae because they contain chlorophyll *a* and their ability to perform oxygenic photosynthesis. However, cyanobacteria are prokaryotes (i.e., no discrete membrane-bound nucleus or membrane-bound subcellular organelles) and are genetically related to other bacteria in the eubacteria domain. Taxonomically, they are classified in the phylum Cyanobacteria or Cyanophyceae (Carmichael 2008; O'Neil et al. 2012).

Cyanobacteria can produce bioactive compounds including toxins, which may be harmful. These biomolecules include hepatotoxic, neurotoxic, and cytotoxic compounds and compounds that can result in allergic reactions (Carmichael 1994; Jaiswal et al. 2008; Volk & Mundt 2007). Studies have also shown that exposure to cyanobacterial cells independent of cyanotoxins can cause health effects; this information is detailed in Appendix D.

Members of *Microcystis*, *Dolichospermum* (*Anabaena*), *Nostoc*, *Oscillatoria*, *Fischerella*, *Planktothrix*, and *Gloeotrichia* can produce microcystins (Carey et al. 2012b; Codd et al. 2005; Duy et al. 2000; Stewart et al. 2006c). *Microcystis* sp. have been documented to occur in blooms on all continents except Antarctica and often dominate phytoplankton assemblages in the summer (O'Neil et al. 2012). Along the margins of Antarctica, other genera of cyanobacteria occur in exposed soils, glaciers, ice shelves, frozen lakes, and stream beds, including *Nostoc*, *Oscillatoria*, *Lyngbya*, or *Synechococcus* (Paerl et al. 2016; Vincent 2007). *Microcystis* sp. have been documented throughout the United States (Carmichael 2001; Jacoby et al. 2000).

Several environmental factors, including nutrient load, increased water temperature, salinity, pH, light intensity, and reduced mixing, provide competitive advantages to *Microcystis* relative to other phytoplankton (Jacoby et al. 2000; Marmen et al. 2016). There is evidence that these environmental factors also affect the relative abundance of microcystin-producing strains and non-microcystin-producing strains (Marmen et al. 2016). *Microcystis* thrives in warmer temperatures, with optimal growth and photosynthesis occurring above 25°C (O'Neil et al. 2012). A Japanese study between May and November 2006 found that the toxin-producing species, *M. aeruginosa*, dominated in months with relatively higher water temperatures, while the non-toxin-producing species, *M. wesenbergii*, dominated in months with lower water temperatures (Imai et al. 2009). Elevated nitrogen and phosphorus inputs may both stimulate *Microcystis* cell growth and biomass accumulation, and can favor microcystin-producing strains (Marmen et al. 2016; O'Neil et al. 2012). During the summer of 1994, *M. aeruginosa* was observed as the dominant species in a toxic bloom in Washington, associated with elevated nitrogen inputs resulting in low nitrogen to phosphorus ratios (Jacoby et al. 2000). The genetic

composition of the bloom can also influence the degree of toxicity associated with an algal bloom. Lee et al. (2015) found that, although *Microcystis* sp. was rarely detected in a shallow lake bloom, most of this population contained the toxin-producing gene. They observed intracellular microcystins at concentrations two to three orders of magnitude greater than extracellular microcystins (Lee et al. 2015).

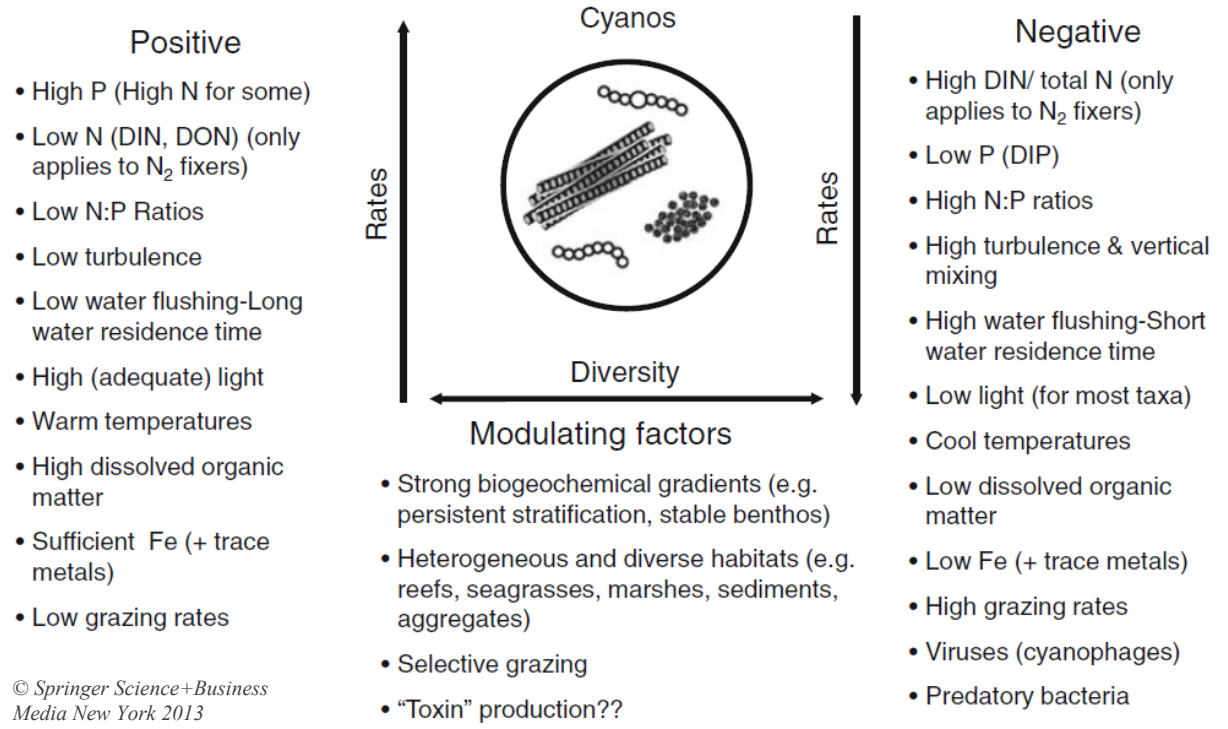
A number of cyanobacterial species including *Cylindrospermopsis raciborskii* (*C. raciborskii*), *Aphanizomenon flos-aquae, Aphanizomenon gracile, Aphanizomenon ovalisporum, Umezakia natans, Anabaena bergii, Anabaena lapponica, Anabaena planctonica, Lyngbya wollei, Rhaphidiopsis curvata,* and *Rhaphidiopsis mediterranea* can produce cylindrospermopsin (B-Béres et al. 2015; Kokocinski et al. 2013; McGregor et al. 2011; Moreira et al. 2013). Cylindrospermopsin-producing cyanobacteria occur in tropical or subtropical regions, but also have been detected in warmer temperate regions. These species do not tend to form visible surface scums and the highest concentrations of cyanobacterial cells occurs below the water surface (Falconer 2005). *C. raciborskii* occurs in freshwater ponds, rivers, reservoirs and eutrophic lakes and has been found in Australia, Asia, Europe, Africa and South, Central and North America (Fuentes et al. 2010). According to a survey conducted in Florida in 1999 from June to November, the most frequently observed toxigenic cyanobacteria were *Microcystis* (43.1 percent), *Cylindrospermopsis* (39.5 percent), and *Anabaena spp.* (28.7 percent) (Burns 2008). In Florida, *C. raciborskii* was found to be the dominant cyanobacteria species in one lake all year round (Burns 2008). In 2006, *C. raciborskii* was detected in lakes in southern Louisiana (Fuentes et al. 2010). Conditions promoting its growth were shallow, warm surface water (over 30°C) and low light intensities. The highest concentrations of *C. raciborskii* were observed from June through August with densities ranging from 37,000 cells/mL to more than 160,000 cells/mL. In a study of two lakes directly connected to Lake Michigan, Hong et al. (2006) found low concentrations only in the late summer, and these were associated with elevated bottom water temperatures and phosphorus concentrations.

Research indicates that cyanotoxins are associated with physiological functions of cyanobacterial cell signaling, nutrient uptake, iron scavenging, maintenance of homeostasis, and protection against oxidative stress and can confer a competitive advantage (Holland & Kinnear 2013). Cylindrospermopsin provides a competitive advantage to cyanobacteria when phosphorus becomes scarce. Bar-Yosef et al. (2010) observed that, when phosphorus is scarce, the cyanobacterium *Aphanizomenon ovalisporum* releases cylindrospermopsin, which causes other microorganisms to release alkaline phosphatase, a compound which will increase available phosphorus. Subsequently, *Aphanizomenon* can gain access to phosphorus made available by other microorganisms while simultaneously conserving the energy and resources required to express and excrete alkaline phosphatase (Bar-Yosef et al. 2010). The precise biological function of microcystin has not been conclusively determined (Zurawell et al. 2005). Studies comparing wild-types and mutants of a microcystin species, examining the genes involved in microcystin biosynthesis, and evaluating *Microcystis* colony size have suggested that microcystins play important physiological roles in cyanobacteria, including colony formation (Kaplan et al. 2012; Zurawell et al. 2005). Although cyanotoxins can negatively affect humans and other animals, research suggests that the primary functions of cyanotoxins are in cyanobacterial physiology and microbial ecology.

A variety of physical, chemical, and environmental factors affect the growth and population dynamics of cyanobacteria, including light intensity, temperature, nutrient

concentrations, biological interactions, and other environmental factors (see summary in Figure 3-1). When the rate of cyanobacterial cell growth exceeds the loss rate for a population, positively buoyant, floating cyanobacterial cells can form a visibly colored scum on the water surface, which can contain more than 10,000 cells/mL (Falconer 1998). The floating scum, as in the case of *Microcystis* species, can be concentrated by prevailing winds in certain surface water areas, especially at the shore. In larger freshwater bodies, such as Lake Erie, these areas of high *Microcystis* concentrations are readily detected by satellite (Stumpf 2014; Wynne et al. 2010). Although these blooms can occur naturally, increasing consensus among scientists is that these blooms have been increasing in recent decades (Carmichael 2008; Hallegraeff 1993; Hudnell 2010).

Figure 3-1. Environmental Factors Influencing Cyanobacterial Bloom Potential in Aquatic Ecosystems, Reproduced from Paerl and Otten (2013b) with Permission of Springer



Nutrients, particularly nutrient over-enrichment, are key environmental drivers that influence the proportion of cyanobacteria in the phytoplankton community, the cyanobacterial biovolume, cyanotoxin production, and the impact that cyanobacteria may have on ecosystem function and water quality (Beaulieu et al. 2013; Paerl et al. 2011). Cyanobacterial toxin concentrations are associated with nutrient levels (Wang et al. 2002); however, different cyanobacteria species use organic and inorganic nutrient forms differently. Loading of nitrogen and/or phosphorus to water bodies from agricultural, industrial, and urban sources influences the development of cyanobacterial blooms and are associated with cyanotoxin production (Paerl et al. 2011). Nitrogen loading can enhance the growth and cyanotoxin levels of *Microcystis* sp. blooms and microcystin synthetase gene expression (Gobler et al. 2007; O'Neil et al. 2012). Gobler et al. (2007) suggested that dominance of toxic *Microcystis* sp. blooms during summer is linked to nitrogen loading, which stimulates growth and cyanotoxin synthesis. This may cause

the inhibition of grazing by mesozooplankton and further accumulation of cyanobacterial cells. Optimal concentrations of total and dissolved phosphorus (Wang et al. 2002) and soluble phosphates and nitrates (ILS 2000; O'Neil et al. 2012; Paerl & Scott 2010; Wang et al. 2010) may also result in the increased production of microcystins. Smith (1983) was the first to describe a strong relationship between the relative amounts of nitrogen and phosphorus in surface waters and toxic cyanobacterial blooms. Smith proposed that cyanobacteria should be superior competitors under conditions of nitrogen-limitation because of their unique capacity for nitrogen fixation, although many cyanobacteria like *Microcystis* species that produce toxins do not fix nitrogen. While the dominance of nitrogen-fixing cyanobacteria at low nitrogen to phosphorus ratios has been demonstrated in mesocosm- and ecosystem-scale experiments in prairie and boreal lakes (Schindler et al. 2008), the hypothesis that low nitrogen to phosphorus ratios favor cyanobacteria formation has been debated and challenged for its inability to reliably predict cyanobacterial dominance (Downing et al. 2001). Eutrophic systems already subject to bloom events are prone to further expansion of these blooms due to additional nitrogen inputs, especially if these nutrients are available from internal sources. As the trophic state increases, aquatic systems absorb higher concentrations of nitrogen (Paerl & Huisman 2008; Paerl & Otten 2013b). Recent surveys of cyanobacterial and algal productivity in response to nutrient pollution across geographically diverse eutrophic lakes, reservoirs, estuarine and coastal waters, and in different experimental enclosures of varying sizes demonstrate that greater stimulation is routinely observed in response to both nitrogen and phosphorus additions. Further, this evidence suggests that nutrient co-limitation is widespread (Elser et al. 2007; Lewis et al. 2011; Paerl et al. 2011). These results strongly suggest that reductions in nutrient pollution are needed to stem eutrophication and cyanobacterial bloom expansion. For example, analysis of observational data collected at larger spatial scales support the idea that controlling total phosphorus and total nitrogen could reduce the frequency of high microcystin events by reducing the biomass of cyanobacteria in the system (Orihel et al. 2012; Scott et al. 2013; Yuan et al. 2014).

The increasing body of laboratory and field data (Carey et al. 2012a; De Senerpont Domis et al. 2007; Huisman et al. 2005; Jeppesen et al. 2009; Kosten et al. 2012; Reynolds 2006; Wagner & Adrian 2009; Weyhenmeyer 2001) suggest that an increase in temperature may influence cyanobacterial dominance in phytoplankton communities. Cyanobacteria may benefit more from warming than other phytoplankton groups due to their higher optimum growth temperatures. The optimum temperatures for microcystin production range from 20 to 25°C (WHO 2003a). The increase in water column stability associated with higher temperatures also may favor cyanobacteria (Carey et al. 2012a; Wagner & Adrian 2009). Kosten et al. (2012) demonstrated that during the summer, the percentage of the total phytoplankton biovolume attributable to cyanobacteria increased steeply with temperature in shallow lakes sampled along a latitudinal transect ranging from subarctic Europe to southern South America. Furthermore, warmer temperatures appear to favor the growth of toxigenic strains of *Microcystis* over non-toxic ecotypes (Dziallas & Grossart 2011; Paerl & Otten 2013b). Indirectly, warming also may increase nutrient concentrations by enhancing mineralization (Gudasz et al. 2010; Kosten et al. 2009; Kosten et al. 2010) by temperature- or anoxia-mediated sediment phosphorus release (Jensen & Andersen 1992; Søndergaard et al. 2003). Thus, temperature may indirectly increase cyanobacterial biomass through its effect on nutrient concentrations. Others have suggested that warmer conditions may raise total phytoplankton biomass through an alteration of top-down regulation by selective grazing that favors larger size phytoplankton species and cyanobacterial blooms (Jeppesen et al. 2009; Jeppesen et al. 2010; Teixeira-de Mello et al. 2009). The

relationship between temperature and cyanobacterial dominance may be explained not only by temperature effect on the competitive advantage of cyanobacteria, but also factors such as the percent area covered and the volume of the lake taken up by submerged macrophytes (Carey et al. 2012a; Kosten et al. 2012). Rising global temperatures and changing precipitation patterns may stimulate cyanobacterial blooms. Warmer temperatures favor surface bloom-forming cyanobacterial genera because they are heat-adapted, and their maximal growth rates occur at relatively high temperatures, often in excess of 25°C (Reynolds 2006; Robarts & Zohary 1987). At these elevated temperatures, cyanobacteria routinely out-compete eukaryotic algae (Elliott 2010; Paerl et al. 2011). Specifically, as the growth rates of the eukaryotic taxa decline in response to warming, cyanobacterial growth rates reach their optima. Warmer surface waters, especially in areas of reduced precipitation, are prone to intense vertical stratification. The strength of vertical stratification depends on the density difference between the warm surface layer and the underlying cold water, which is influenced by the amount of precipitation. As temperatures rise due to climate change, stratification is expected to occur earlier in the spring and persist longer into the fall (Paerl & Otten 2013b). The increase in water column stability associated with higher temperatures and climate change may therefore favor cyanobacteria production and possibly the prevalence of cyanotoxins such as microcystins (Carey et al. 2012a; Wagner & Adrian 2009).

Sunlight availability and turbidity can have a strong influence on the cyanobacteria species that predominate, as well as the depth at which they occur (Carey et al. 2012a; Falconer 2005). For example, *Microcystis aeruginosa* occurs mostly at the surface with higher light intensities and in shallow lakes. Kosten et al. (2012) surveyed 143 shallow lakes along a latitudinal gradient (between 5–55°S and 38–68°N) from subarctic Europe to southern South America. Their analyses found a greater proportion of the total phytoplankton biovolume attributable to cyanobacteria in lakes with high rates of light absorption. Kosten et al. (2012) could not establish cause and effect from these field data, but other controlled experiments and field data have demonstrated that light availability can affect the competitive balance among a large group of shade-tolerant species of cyanobacteria, mainly *Oscillatoriales* and other phytoplankton species (Scheffer et al. 1997; Smith 1986). Overall, results from Kosten et al. (2012) suggest that higher temperatures interact with nutrient loading and underwater light conditions in determining the proportion of cyanobacteria in the phytoplankton community in shallow lakes.

Cyanobacterial blooms have been shown to intensify and persist at pH levels between six and nine (WHO 2003a). When these blooms are massive or persist for a prolonged period, they can become harmful. Kosten et al. (2012) noted the impact of pH on cyanobacteria abundance in lakes along a latitudinal transect from Europe to southern South America. The percentage of cyanobacteria in the 143 shallow lakes sampled was highly correlated with pH, with an increased proportion of cyanobacteria at higher pH. Cyanobacteria have a competitive advantage over other phytoplankton species because they are efficient users of carbon dioxide in water (Caraco & Miller 1998; Shapiro 1984). This characteristic is especially advantageous for cyanobacteria under conditions of higher pH when the concentration of carbon dioxide in the water column is diminished due to photosynthetic activity. Although this could explain the positive correlation observed between pH and the proportion of cyanobacteria, the high proportion of cyanobacteria at high pH could be the result of an indirect nutrient effect as described previously (see discussion in Temperature section). As photosynthesis intensifies, pH increases due to carbon dioxide uptake by algae, resulting in a shift in the carbonic buffer equilibrium and a higher

concentration of basic forms of carbonate. Thus, higher water column pH may be correlated with a higher proportion of cyanobacteria because of higher photosynthetic rates, which can be linked with high nutrient concentrations (Duy et al. 2000) that stimulate phytoplankton growth and bloom formation. High iron concentrations (more than 100 µM) have also been shown to increase cyanobacterial cell density and chlorophyll content in *Microcystis aeruginosa* (Kosakowska et al. 2007).

Cyanobacterial blooms commonly occur from spring to early fall in various regions of the United States (Wynne & Stumpf 2015). Cyanobacteria take advantage of conditions that can occur in late summer and early fall such as elevated water temperatures and increased vertical stratification in lakes and reservoirs (Paerl & Huisman 2008). Some blooms occur later in summer and early fall. Vertical biomass structure and cyanotoxin production can be influenced by seasonal changes as well as severe weather conditions (e.g., strong wind or rainfall) and also by runoff. At times, the hypolimnion (bottom layer of the water column) can have a higher cyanobacteria biomass and display different population dynamics than the epilimnion (upper layer of the water column). Conversely, seasonal effects of increasing temperatures and changes in wind patterns may favorably influence the upper water column cyanobacterial community. This vertical variability is common and attributed to four causes, each of which may occur at different times, including: (a) sinking of dead/dying cyanobacterial cells; (b) density stratification of the water column, especially nutrient concentrations and light, which affects all aspects of cyanobacteria growth; (c) increased nutrient supply from organic-rich bottom sediment (even when the water body is not density-stratified), encouraging cyanobacteria growth at or near the bottom sediment; and (d) species-specific factors such as the tendency to form surface scums in the case of *M. aeruginosa* or the presence of resting spores in the sediment in the case of *N. spumigena* (Drake et al. 2010).

In addition to occurrence in lakes and reservoirs, cyanobacteria and cyanotoxins have been detected in flowing rivers and streams (Chaney 2016; Commonwealth of Kentucky: Energy and Environment Cabinet 2015; Florida Department of Environmental Protection 2016; Loftin et al. 2016a; Otten et al. 2015; Paerl & Otten 2013b; Parker 2016). In some cases, the source of the cyanobacteria can be traced to an upstream water body such as a lake or reservoir. In 2016, a bloom in Lake Okeechobee impacted the St. Lucie River and estuary and the Caloosahatchee River and estuary in Florida (Florida Department of Environmental Protection 2016). Otten et al. (2015) used microbial source tracking techniques to trace the source of a toxic *Microcystis* bloom in the Klamath River in Oregon to a single upstream reservoir. Their results showed that large quantities of cyanobacterial cells can withstand passage through hydroelectric installations and transport over 300 kilometers. Cyanobacterial bloom development has been documented near dams and man-made reservoirs (Chaney 2016; Giannuzzi et al. 2011; Otten et al. 2015; Sierosławska et al. 2010). Environmental characteristics including nutrients and flow rate can affect phytoplankton dynamics (Paerl & Otten 2013b). Zhang et al. (2015) observed that low flow conditions favored cyanobacteria and higher flow conditions favored green algae.

Cyanobacterial blooms can also occur in rivers and streams without a known lake or reservoir source in the water column or as part of the benthic community (Commonwealth of Kentucky: Energy and Environment Cabinet 2015; Loftin et al. 2016a). Loftin et al. (2016a) suggest that low stream flow, shallow depth, and high water column light penetration in Piedmont streams favored periphyton occurrence (mixture of algae, cyanobacteria, heterotrophic bacteria, and detritus). A review by Quiblier et al. (2013) of benthic freshwater cyanobacterial

ecology found that nutrients, flow regime, wave action, climate, and geology can influence benthic cyanobacterial community composition and suggest that due to a high desiccation tolerance, cyanobacteria occurrence in benthic mat communities is also a concern in ephemeral streams.

In addition, there are microbial interactions that may occur within blooms, such as competition and adaptation between toxic and nontoxic cyanobacterial strains, as well as impacts from viruses and zooplankton grazers like *Daphnia* (large generalist grazers), copepods, and cladocerans (Ger et al. 2014). Each of these microbial-related factors can cause fluctuations in bloom development and composition.

In summary, there is a complex interplay of environmental factors that dictates the spatial and temporal changes in the concentration of cyanobacterial cells and their toxins with respect to the dominant species as illustrated in Figure 3-1 (Paerl & Otten 2013b). Factors such as the amount and timing of nutrient supply (i.e., nutrient concentration and nutrient loading), the relative proportions of nutrients (i.e., nitrogen to phosphorus ratio), dissolved organic matter availability, temperature, and light attenuation, as well as other physico-chemical processes, can play a role in shaping cyanobacterial bloom composition and cyanotoxin production (Paerl & Huisman 2008; Paerl & Otten 2013b). Phytoplankton competition and food web interactions that occur as blooms develop, persist, and decline can also impact cyanotoxin concentrations in surface waters. In addition, impacts of climate change, including potential warming of surface waters and changes in precipitation, could result in changes in ecosystem dynamics that lead to more frequent formation of cyanobacteria blooms and their associated toxins (Paerl & Huisman 2008; Paerl & Otten 2013b; Paerl et al. 2011).

3.2 Cyanotoxins

Much of the information and the studies summarized in this section for microcystin and cylindrospermopsin are described in detail in EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* and *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (HESDs), and EPA's *Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins* and *Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin* (Drinking Water Health Advisories) (U.S. EPA 2015a; U.S. EPA 2015b; U.S. EPA 2015c; U.S. EPA 2015d).

3.2.1 Chemical and Physical Properties

Summary information for chemical and physical properties is provided in this section. Additional information can be found in the EPA's HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c; U.S. EPA 2015d).

Structurally, microcystins are monocyclic heptapeptides that contain seven amino acids joined end-to-end and then head to tail to form cyclic compounds that are comparatively large; molecular weights range from approximately 800 to 1,100 g/mole. The cyclic peptides include more than 100 congeners of microcystins (Niedermeyer 2014). Figure 3-2 provides the structure of microcystin where X and Y represent variable amino acids. Although substitutions mostly occur in positions X and Y, other modifications have been reported for all of the amino acids (Puddick et al. 2015).

The microcystins are named based on their two variable amino acids (Carmichael et al. 1988). For example, microcystin-LR, the most common congener, contains leucine (L) and arginine (R) (Carmichael 1992). The letters used to identify the variable amino acids are the standard single letter abbreviations for the amino acids found in proteins. The variable amino acids are usually the L-amino acids as found in proteins. For example, microcystin-LR is for the microcystin with leucine in the X position of Figure 3-2 and arginine in the Y position in Figure 3-2. Table 3-1 lists the most common microcystins congeners.

Figure 3-2. Structure of Microcystin (Kondo et al. 1992)

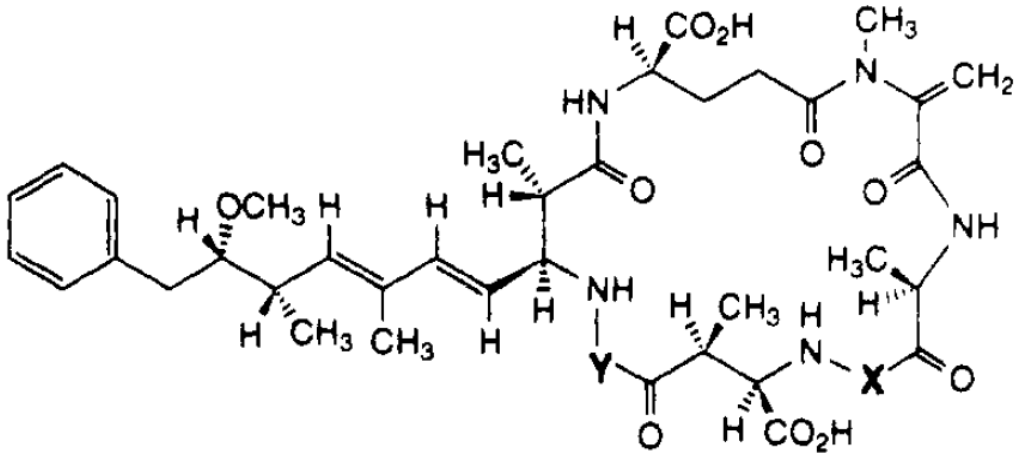


Table 3-1. Abbreviations for Microcystins (Yuan et al. 1999)

Microcystin Congeners	Amino Acid in X	Amino Acid in Y
Microcystin-LR	Leucine	Arginine
Microcystin-RR	Arginine	Arginine
Microcystin-YR	Tyrosine	Arginine
Microcystin-LA	Leucine	Alanine
Microcystin-LY	Leucine	Tyrosine
Microcystin-LF	Leucine	Phenylalanine
Microcystin-LW	Leucine	Tryptophan

The preponderance of toxicological data on the effects of microcystins is restricted to the microcystin-LR congener. Toxicity data suggest that microcystin-LR is as potent as or more potent than other studied microcystins and that the most toxic microcystins are those with the more hydrophobic L-amino acids (-LA, -LR, -YR, and -YM); the least toxic are those with hydrophilic amino acids, such as microcystin-RR. Data on the -RR, -YR, and -LA congeners, however, are limited, and toxicity values cannot be derived for them. Values developed from data specific to microcystin-LR are considered applicable to and appropriate for individual and mixtures of microcystin congeners.

Table 3-2 provides chemical and physical properties of microcystin-LR. Microcystins are water-soluble. In aquatic environments, the cyclic peptides tend to remain contained within the cyanobacterial cell and are released in substantial amounts only upon cyanobacterial cell lysis.

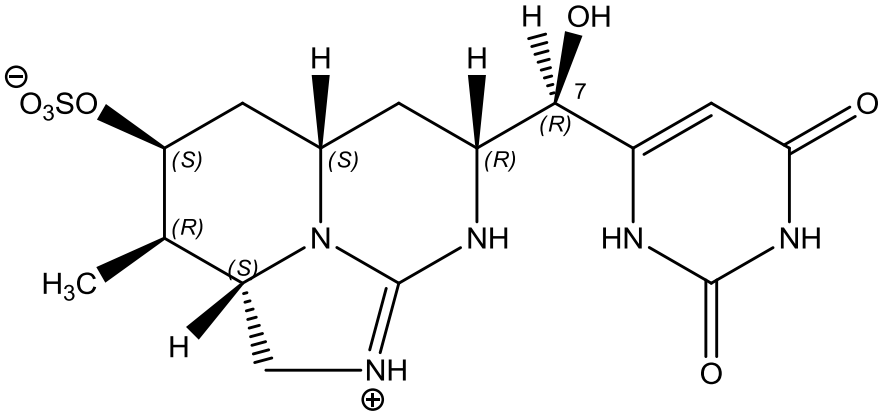
Table 3-2. Chemical and Physical Properites of Microcystin-LR

Property	Microcystin-LR
Chemical Abstracts Registry (CAS) Number	101043-37-2
Chemical Formula	$C_{49}H_{74}N_{10}O_{12}$
Molecular Weight	995.17 g/mole
Color/Physical State	Solid
Boiling Point	N/A
Melting Point	N/A
Density	1.29 g/cm^3
Vapor Pressure at 25°C	N/A
Henry's Law Constant	N/A
Log K_{ow}	2.16
K_{oc}	N/A
Solubility in Water	Highly
Other Solvents	Ethanol and methanol

Sources: Chemical Book (2012); TOXLINE (2012); Ward and Codd (1999) for log K_{ow}.

Cylindrospermopsin is a tricyclic alkaloid with the following molecular formula $C_{15}H_{21}N_5O_7S$ (Ohtani et al. 1992) and a molecular weight of 415.43 g/mole. It is zwitterionic (i.e., a dipolar ion with localized positive and negative charges) (Ohtani et al. 1992). The chemical structure of cylindrospermopsin is presented in Figure 3-3. Additional congeners and analogs have been identified; see U.S. EPA (2015b; 2015c) for more information.

Figure 3-3. Structure of Cylindrospermopsin (de la Cruz et al. 2013)



The physical and chemical properties of cylindrospermopsin are presented in Table 3-3. Cylindrospermopsin is highly soluble in water (Chiswell et al. 1999; Moore et al. 1998). Cylindrospermopsin is isolated for commercial use mostly from *C. raciborskii*. Many of the physicochemical properties of cylindrospermopsin in the environment such as vapor pressure and boiling and melting points are unknown.

Table 3-3. Chemical and Physical Properties of Cylindrospermopsin

Property	Cylindrospermopsin
Chemical Abstracts Service (CAS) Registry Number	143545-90-8
Chemical Formula	$C_{15}H_{21}N_5O_7S$
Molecular Weight	415.43 g/mole
Color/Physical State	white powder
Boiling Point	N/A
Melting Point	N/A
Density	2.03 g/cm^3
Vapor Pressure at 25°C	N/A
Henry's Law Constant	N/A
K_{ow}	N/A
K_{oc}	N/A
Solubility in Water	Highly
Other Solvents	Dimethyl sulfoxide (DMSO) and methanol

Sources: Chemical Book (2012); TOXLINE (2012).

3.2.2 Sources and Occurrence

Cyanobacterial density in a bloom and cyanotoxin concentration are not always closely related. Cyanotoxin concentrations depend on the dominance and diversity of species and strains within the bloom along with environmental and ecosystem influences on bloom dynamics (Chorus et al. 2000; Hitzfeld et al. 2000; WHO 1999). Cyanotoxin production by cyanobacteria is highly variable and strongly influenced by the environmental conditions. It can vary among strains and clones of a single species (Carmichael 1994; Utkilen & Gjølme 1992) and within and between blooms (Codd & Bell 1985). Growth phase also can influence cyanotoxin production (Jaiswal et al. 2008). Although studies of the impact of environmental factors on cyanobacteria bloom are ongoing, a variety of factors can influence cyanotoxin production, including nutrient (nitrogen, phosphorus) concentrations, light levels, temperature, pH, oxidative stressors, and interactions with other biota (viruses, bacteria, and animal grazers), and the combined effects of these factors (Paerl & Otten 2013a; Paerl & Otten 2013b). Factors discussed previously that influence cyanobacterial growth can also influence cyanotoxin production, however, growth and toxin production do not necessarily coincide. Recent research by Francy et al. (2016) on modeling the relationship of environmental variables and cyanotoxin levels has shown that

certain environmental factors may be useful to estimating microcystin concentrations above a threshold level.

The proportion of intracellular versus extracellular cyanotoxin can also vary. Extracellular microcystins (either dissolved in water or bound to other materials) typically make up less than 30 percent of the total microcystin concentration in source water (Graham et al. 2010). Most of the microcystins are intracellular and released into the water when the cyanobacterial cells rupture or die. Cylindrospermopsin may be retained within the cyanobacterial cell or released. The ratio of intracellular to extracellular cyanotoxin can change depending on the growth phase with as much as 50 percent of cylindrospermopsin produced by *C. raciborskii* released extracellularly (Griffiths & Saker 2003).

3.2.2.1 Surface Water

Microcystins

Microcystins are the most common cyanotoxins found worldwide and have been reported in surface waters in most of the states in the United States (Funari & Testai 2008). Dry-weight concentrations of microcystins in surface freshwater cyanobacterial blooms or surface freshwater samples reported worldwide between 1985 and 1996 ranged from 1 to 7,300 μg/g. Water concentrations of extracellular plus intracellular microcystins ranged from 0.04 to 25,000 μg/L. The concentration of extracellular microcystins ranged from 0.02 to a high of 1,800 μg/L reported following treatment of a large cyanobacteria bloom with algaecide (WHO 1999), and the U.S. Geological Survey (USGS) reported a concentration of 150,000 μg/L total microcystins in a lake in Kansas (Graham et al. 2012).

Microcystins have been detected in most states, and over the years, many studies have been done to determine their occurrence in surface water. The remainder of this section provides examples of microcystin occurrence observations throughout the United States.

According to a survey conducted in Florida in 1999 between the months of June and November, the most frequently observed cyanobacteria were *Microcystis* (43.1 percent), *Cylindrospermopsis* (39.5 percent), and *Anabaena* spp. (28.7 percent) (Burns 2008). Of 167 surface water samples taken from 75 waterbodies, microcystin was the most commonly found cyanotoxin in water samples collected, occurring in 87 water samples.

In 2002, the Monitoring and Event Response to Harmful Algal Blooms in the Lower Great Lakes project evaluated the occurrence and distribution of cyanotoxins in the lower Great Lakes region (Boyer 2007). Analysis for total microcystins was performed using Protein Phosphatase Inhibition Assay. Microcystins were detected in at least 65 percent of the samples, mostly in Lake Erie, Lake Ontario, and Lake Champlain. The National Oceanic and Atmospheric Administration Center of Excellence for Great Lakes and Human Health continues to monitor the Great Lakes and regularly samples cyanobacterial blooms for microcystin in response to bloom events.

A 2004 study of the Great Lakes found high levels of cyanobacteria during the month of August (Makarewicz et al. 2006). Microcystin-LR was analyzed by protein phosphatase inhibition assay (limit of detection of 0.003 μg/L) and was detected at levels of 0.084 μg/L in the nearshore and 0.076 μg/L in the bays and rivers. This study reported higher levels of microcystin-LR (1.6 to 10.7 μg/L) in smaller lakes in the Lake Ontario watershed.

In 2006, USGS conducted a study of 23 lakes in the Midwestern United States in which cyanobacterial blooms were sampled to determine the co-occurrence of cyanotoxins in cyanobacterial blooms (Graham et al. 2010). This study reported that microcystins were detected in 91 percent of the lakes sampled with 17 percent of microcystin-positive samples exceeding 20 µg/L. Mixtures of all the microcystin congeners measured (-LA, -LF, -LR, -LW, -LY, -RR, and -YR) were common, and all the congeners were present in association with the blooms. Microcystin-LR and -RR were the dominant congeners detected with mean concentrations of 104 and 910 µg/L respectively.

In 2007, the NLA conducted the first national probability-based survey of the condition of the nations' lakes, ponds, and reservoirs (U.S. EPA 2009). This baseline study provided estimates of the condition of natural and man-made freshwater lakes, ponds, and reservoirs greater than 4 hectares (10 acres) and at least one meter deep. The NLA measured microcystins using enzyme linked immunosorbent assays (ELISA) with a detection limit of 0.1 µg/L as well as cyanobacterial cell counts and chlorophyll *a* concentrations, which were indicators of the presence of cyanotoxins. Samples were collected in open water at mid-lake. Due to the design of the survey, no samples were taken nearshore or in other areas where scums were present. These surveys covered a total of 1,028 lakes, which represented nearly 50,000 lakes in the conterminous United States. This assessment found that cyanobacteria were detected in almost all lakes (U.S. EPA 2009). Cyanobacteria were the dominant member of the phytoplankton community in 76 percent of lake samples. Subsequent analysis indicated that potential microcystin-producing species occurred in 95 percent of samples (Loftin et al. 2016b).

Microcystins are the most commonly detected class of cyanotoxin and have been found in lakes in the contiguous United States (U.S. EPA 2009) and streams in the Southeastern United States (Loftin et al. 2016b). Microcystins were present in 30 percent of the lakes sampled nationally by the NLA, with sample concentrations that ranged from the limit of detection (0.1 µg/L) to 225 µg/L (U.S. EPA 2009). Microcystins were detected in in 32 percent of lake water samples with a mean concentration of 3.0 µg/L (based on detections only) and microcystin concentrations above the WHO thresholds of concern of 10 and 20 µg/L were present in 1.1 percent of samples nationally (Loftin et al. 2016b). States with lakes reporting microcystins levels above > 10 µg/L are shown in Table 3-4. NLA data show two states (North Dakota and Nebraska) had 9 percent of samples above 10 µg/L. Other states including Iowa, Texas, South Dakota, and Utah also had samples that exceeded 10 µg/L. Several NLA samples in North Dakota, Nebraska, and Ohio exceeded 20 µg/L (192 and 225 µg/L respectively). EPA completed a second survey of lakes in 2012, however, those data have not yet been released.

USGS did a study in the Upper Klamath Lake in Oregon in 2007 and detected total microcystin concentrations between 1 µg/L and 17 µg/L (VanderKooi et al. 2010). USGS also monitored Lake Houston in Texas from 2006 to 2008, and found microcystin in 16 percent of samples and at concentrations less than or equal to 0.2 µg/L (Beussink & Graham 2011). In 2011, USGS conducted a study on the upstream reservoirs of the Kansas River to characterize the transport of cyanobacteria and associated compounds (Graham et al. 2012). Concentrations of total microcystin were low in the majority of the tributaries with the exception of Milford Lake, which had higher total microcystin concentrations, some exceeding the Kansas

Table 3-4. States Surveyed as Part of the 2007 National Lakes Assessment with Water Body Microcystins Concentrations above 10 µg/L (U.S. EPA 2009)

State	Number of Sites Sampled	Percentage of Samples with Detection of Microcystins > 10 µg/L	Maximum Detection of Microcystins
North Dakota	38	9.1 percent	192 µg/L
Nebraska	42	9.1 percent	225 µg/L
South Dakota	40	4.9 percent	33 µg/L
Ohio	21	4.5 percent	78 µg/L*
Iowa	20	4.5 percent	38 µg/L*
Utah	26	3.6 percent	15 µg/L*
Texas	51	1.8 percent	28 µg/L*

*Single Sample

recreational guideline level of 20 µg/L. Upstream from Milford Lake, a cyanobacterial bloom was observed with a total microcystin concentration of 150,000 µg/L. When sampled a week later, total microcystin concentrations were less than 1 µg/L. The study authors indicated that this may be due to dispersion of microcystins through the water column or to other areas, or by degradation of microcystins via abiotic and biological processes. Samples taken during the same time from outflow waters contained total microcystin concentrations of 6.2 µg/L.

In 2005, Washington State Department of Ecology developed the Ecology Freshwater Algae Program to focus on the monitoring and management of cyanobacteria in Washington lakes, ponds, and streams (WSDE 2012). The data collected have been summarized in a series of reports for the Washington State Legislature (Hamel 2009; Hamel 2012). Microcystin levels ranged from the detection limit (0.05 µg/L) to 4,620 µg/L in 2008, to 18,700 µg/L in 2009, to 853 µg/L in 2010, and to 26,400 µg/L in 2011.

A survey conducted during the spring and summer of 1999 and 2000 in more than 50 lakes in New Hampshire found measureable microcystin concentrations in all samples (Haney & Ikawa 2000). Microcystins were analyzed by ELISA and were found in all of the lakes sampled with a mean concentration of 0.1 µg/L. In 2005 and 2006, a study conducted in New York, including Lake Ontario, found variability in microcystin-LR concentrations within the Lake Ontario ecosystem (Makarewicz et al. 2009).

Since 2007, Ohio EPA (2012) has been monitoring inland lakes for cyanotoxins. Of the 19 lakes in Ohio sampled during the NLA, 36 percent had detectable levels of microcystins. In 2010, Ohio EPA sampled Grand Lake, St. Marys for anatoxin-a, cylindrospermopsin, microcystins, and saxitoxin. Microcystin levels ranged from below the detection limit (< 0.15 µg/L) to more than 2,000 µg/L. Follow-up samples taken in 2011 for microcystins indicated concentrations exceeded 50 µg/L in August. During the same month, sampling in Lake Erie found microcystins levels exceeding 100 µg/L.

In 2008, NOAA began monitoring for cyanobacterial blooms in Lake Erie using high temporal resolution satellite imagery. Between 2008 and 2010, *Microcystis* cyanobacterial blooms were associated with water temperatures above 18°C (Wynne et al. 2013). Using the

Great Lakes Coastal Forecast System, forecasts of bloom transport are created to estimate the trajectory of the bloom, and these are distributed as bulletins to local managers, health departments, researchers, and other stakeholders. To evaluate bloom toxicity, the Great Lakes Environmental Research Laboratory collected samples at 6 to 8 stations each week for 24 weeks, measuring cyanotoxin concentrations as well as chlorophyll biomass and an additional 18 parameters (e.g., nutrients) to improve future forecasts of these blooms. Microcystins can be separated into particulate (cell-bound) and dissolved (extracellular) phases, which can be measured by testing concentrations in the filter and filtrate fractions of the sampled water (Graham & Jones 2007; Zastepa et al. 2014). In 2014, particulate microcystin concentrations ranged from below detection to 36.7 μg/L. Samples taken in 2015 and 2016 showed particulate microcystin concentration ranges from below detection to 9.19 μg/L and from below detection to 21.26 μg/L, respectively. Particulate microcystin concentrations peaked in August 2014 at all sites, with the Maumee Bay site yielding the highest concentration of the entire three-year sampling period. Dissolved microcystin concentrations were also collected at each site in 2014 from September until the end of the sampling period in November, as well as during the field sampling seasons in 2015 and 2016. During the final months of sampling in 2014 (October to November), dissolved microcystin concentrations were detected with peak concentrations of 0.8 μg/L (mean: 0.28 +/- 0.2 μg/L) whereas particulate microcystin concentrations were below detection limits on many dates, indicating that a majority of the microcystin (mean: 72 percent +/- 37 percent) were in the dissolved form, as the bloom declined in intensity. Measured dissolved microcystin concentrations in the following two years ranged from levels below detection to peaks of 0.69 μg/L in September 2015 and 1.76 μg/L in July 2016 (NOAA 2014). Note that the health-protective value for microcystins recommended in this document should be compared with the total microcystins detected and not delineated between intracellular or extracellular microcystin. Cells containing microcystin can be swallowed while recreating and contribute to the overall exposure to the toxin.

Two notable cyanobacterial blooms occurred in Florida and Utah in 2016, resulting in microcystin detections. From July 14 to September 14, an extensive cyanobacterial bloom covering 100 square miles occurred in Utah Lake, Jordan River, and nearby canals and included the cyanobacterial genera *Geitlerinema, Oscillatoria*, and *Pseudanabaena* (Utah Department of Environmental Quality 2016). Microcystin concentrations ranged from < 0.5–176 μg/L. The Utah Department of Environmental Quality reported over 500 human exposures with 30 percent of these cases reporting symptoms such as gastrointestinal distress, headache, and eye and skin irritation. In addition, 27 animal exposures were reported (Utah Department of Environmental Quality 2016).

In 2016, a 239-square mile cyanobacterial bloom in Lake Okeechobee, Florida, and downstream waterways resulted in a state of emergency in four counties on the Gulf and Atlantic coasts of Florida (Chaney 2016; Parker 2016). From May 4 to August 4, the Florida Department of Environmental Protection took approximately 200 water samples from the St. Lucie River and estuary, Caloosahatchee River and estuary, Lake Okeechobee, Indian River Lagoon, and other nearshore marine locations (Florida Department of Environmental Protection 2016). Microcystin concentrations ranged from below the detection limit to 414.3 μg/L. Among the species identified were *Microcystis aeruginosa*, *Scrippsiella trochoidea*, *Planktolyngbya limnetica*, *Dolichospermum circinalis*, and *Plectonema wollei* (Florida Department of Environmental Protection 2016). Lake Okeechobee, located north of the Everglades, is the largest freshwater lake in Florida. It is subject to agricultural runoff from adjacent cattle farms and sugar cane

fields, which contributed to the formation of this massive cyanobacterial bloom (Parker 2016). Water may be pumped out of the lake to the coast through the St. Lucie River and the Caloosahatchee River to prevent the lake level from rising too high after periods of heavy rain, (Parker 2016). As a result of the microcystin levels and visible cyanobacterial scum from water discharged from the lake that flowed downstream to coastal areas, beaches along the Atlantic were closed, and a state of emergency was declared in the counties of Martin, St. Lucie, Palm Beach, and Lee (Chaney 2016; Florida Department of Environmental Protection 2016).

Cylindrospermopsin

As noted above, EPA's NLA conducted the first national probability-based survey of lakes (U.S. EPA 2009) and published results in 2007. USGS subsequently analyzed the stored samples collected and detected cylindrospermopsin in 4 percent of samples, with a mean concentration 0.56 µg/L and a range from the limit of detection, 0.01 µg/L, to a maximum of 4.4 µg/L (Loftin et al. 2016b). Potential cylindrospermopsin-producing species occurred in 67 percent of samples (Loftin et al. 2016b). In general, fewer surface water occurrence data are available for cylindrospermopsin compared to microcystin. This is likely because during blooms, testing for microcystin is much more common than testing for cylindrospermopsin.

USGS also detected cylindrospermopsin in 9 percent of blooms sampled during a 2006 USGS survey of 23 lakes in the Midwestern United States (Graham et al. 2010). The low concentrations of cylindrospermopsin detected (0.12 to 0.14 µg/L) in the study occurred in bloom communities dominated by *Aphanizomenon* or *Anabaena* and *Microcystis*.

Cylindrospermopsin has been detected in lakes throughout multiple states. In a 1999 study, cylindrospermopsin was detected in 40 percent of 167 water samples taken from 87 water bodies in Florida during the months of June and November (Burns 2008). However, the actual cylindrospermopsin concentrations were not reported. In 2005, the U.S. Army Corps of Engineers detected cylindrospermopsin at a maximum concentration of 1.6 µg/L in lake water samples from Oklahoma (Lynch & Clyde 2009). In Grand Lake in St. Marys, Ohio, cylindrospermopsin concentrations as high as 9 µg/L were reported in 2010 (Ohio EPA 2012).

3.2.2.2 Ambient Air

According to Wood and Dietrich (2011), waterborne cyanotoxins can be aerosolized through a bubble-bursting process, in which the cyanobacteria and cyanotoxins are ejected and carried into the air by the resulting droplets from the bubble bursting. Microcystin that is free or bound to particles can be deposited into the deepest bronchiolar or alveolar cavities; the cyanobacterial cells can be likely deposited in the upper respiratory tract (Wood & Dietrich 2011).

Four studies provide air concentration data indicating that recreational surface waters with cyanotoxin-producing cyanobacterial blooms can result in aerosolized cyanotoxins. Backer et al. (2008) used personal air samplers in a 3-day study of recreational activities in a lake with a cyanobacterial bloom, either carried by the study participant or placed on the participant's boat. The microcystin concentrations in air ranged from below the limit of detection (0.0037 ng/m^3) to 0.456 ng/m^3. Backer et al. (2010) also detected microcystins in ambient air for one day, at one lake, and only from the shoreline sampler. The average air concentration was 0.052 ng/m^3. They also collected 44 personal air samples, which ranged from the limit of detection (0.1 ng/m^3) to

0.4 ng/m^3. The authors noted that the daily mean concentrations of microcystin in personal air samples did not correlate with the concentrations of *Microcystis* cells, dissolved microcystin, or total microcystin in the sampled lake water.

Wood and Dietrich (2011) studied Lake Rotorua (New Zealand) when it was experiencing a dense bloom of microcystin-producing *Microcystis* species. They measured a maximum microcystin concentration in the water of (2,140 µg/L) and air concentrations from 0.0003 to 0.0018 ng/m^3.

Cheng et al. (2007) used high volume and personal air samplers to measure microcystins in the air in at a lake with a cyanobacterial bloom. They measured low concentrations of microcystin in the water (approximately 1 µg/L) and air concentrations ranging from below the detection limit (0.02 ng/m^3) to 0.08 ng/m^3.

3.2.2.3 Other Sources of Microcystins and Cylindrospermopsin

Extracts from *Arthrospira* (*Spirulina* spp.) and *Aphanizomenon flos-aquae* have been used as dietary blue-green algae supplements (BGAS) (Funari & Testai 2008). These supplements are reported to have beneficial health effects including supporting weight loss, and increasing alertness, energy and mood elevation for people suffering from depression (Jensen et al. 2001). A study suggested that BGAS can be contaminated with microcystins ranging from 1 µg/g up to 35 µg/g (Dietrich & Hoeger 2005). Heussner et al. (2012) analyzed 18 commercially available BGAS for the presence of cyanotoxins. All products containing *Aphanizomenon flos-aquae* tested positive for microcystins at levels ≤ 1 µg microcystin-LR equivalents/g dry weight. Cylindrospermopsin was not found in any of the supplements.

3.2.3 Environmental Fate

Different physical and chemical processes are involved in the persistence, breakdown, and movement of microcystins and cylindrospermopsin in aquatic systems as described below.

3.2.3.1 Mobility

Cyanotoxins can move within water systems or they can be transported between systems. Mechanisms concentrating cyanobacterial cells can also act to concentrate their cyanotoxins, leading to negative human health impacts including impacts on surface waters and direct contact and aerosol exposure (Bláha et al. 2009; Carmichael 2001; Cheung et al. 2013; Codd et al. 2005).

Microcystins may adsorb onto naturally suspended solids and dried crusts of cyanobacteria. They can precipitate out of the water column and reside in sediments for months (Falconer 1998; Han et al. 2012). Ground water is generally not expected to be at risk of cyanotoxin contamination, however, ground water under the direct influence of surface water can be vulnerable. A study conducted by the USGS and the University of Central Florida determined that microcystin and cylindrospermopsin did not sorb in sandy aquifers and were transported along with ground water (O'Reilly et al. 2011). The authors suggested that the removal of microcystin was due to biodegradation.

In sediments, cylindrospermopsin exhibits some adsorption to organic carbon, with little adsorption observed on sandy and silt sediments (Klitzke et al. 2011). The low adsorption of

cylindrospermopsin reduces its residence time in sediments, thus reducing the opportunity for microbial degradation.

3.2.3.2 Persistence

Microcystins

Microcystins are relatively stable and resistant to chemical hydrolysis or oxidation at or near neutral pH. Elevated or low pH or temperatures above 30°C may cause slow hydrolysis. Microcystins have been observed to persist for 21 days to 2–3 months in solution and up to 6 months in dry scum (Funari & Testai 2008; Rapala et al. 2006). Environmental conditions such as temperature, pH, presence of light, salinity, and presence of certain aquatic bacteria, can influence the rate of microcystin degradation (Schmidt et al. 2014). Microcystins can persist even after a cyanobacterial bloom is no longer visible (Lahti et al. 1997b; Zastepa et al. 2014). In a study by Zastepa (2014), dissolved microcystin-LA was present at a concentration of 20 μg/L or greater for 9.5 weeks even though the *Microcystis* bloom was not visible after 5 weeks.

In the presence of full sunlight, microcystins undergo photochemical breakdown, but this varies by microcystin congener (Chorus et al. 2000; WHO 1999). Zastepa et al. (2014) suggest that microcystin-LA degrades at a slower rate than microcystin-LR, -RR, and -YR congeners. The presence of water-soluble cyanobacterial cell pigments, in particular phycobiliproteins, enhances this breakdown. Breakdown can occur in as few as 2 weeks to longer than 6 weeks, depending on the concentration of pigment and the intensity of the light (Tsuji et al. 1994; Tsuji et al. 1995). Several other factors, including photosensitizer concentration, pH, wavelength of light (Schmidt et al. 2014), and whether microcystins are dissolved or present in particulate matter (Lahti et al. 1997b) can affect the rate of transformation or photodegradation. According to Tsuji et al. (1994) and Tsuji et al. (1995), microcystin-LR was photodegraded with a half-life of about 5 days in the presence of 5 mg/L of extractable cyanobacterial pigment. Humic substances can also act as photosensitizers and can increase the rate of microcystin breakdown in sunlight. Others have found that high concentrations of humic acids can slow the rate of microcystin transformation by sunlight (Schmidt et al. 2014). In deeper or turbid water, the breakdown rate is slower. Welker and Steinberg (2000) estimated the maximum rate of microcystin-LR degradation in the presence of humic substance photosensitizers. Extrapolating results from their small experimental tubes to a water column of 1 meter, Schmidt et al. (2014) estimated the half-life of microcystin-LR to be 90 to 120 days per meter of water depth in surface waters. The researchers also demonstrated that the wavelength of light can also affect degradation rates; complete microcystin degradation has been observed within 1 hour when exposed to 254-nm light and within 5 days using 365-nm light. According to Lahti et al. (1997b), microcystin-LR follows first-order decay kinetics, with a decimal reduction time of 30 days for dissolved microcystins compared with 15 days for microcystins found in particulate matter. Zastepa et al. (2014) also found that dissolved microcystin-LA persists longer than microcystin-LA in particulates, with *in situ* half-lives of 15.8 days and 6.5 days, respectively.

Microcystins are susceptible to degradation by aquatic bacteria found naturally in surface waters (Jones et al. 1994). Bacteria isolates of *Arthrobacter, Brevibacterium, Rhodococcus, Paucibacter*, and various strains of the genus *Sphingomonas* (*Pseudomonas*) have been reported to be capable of degrading microcystin-LR (de la Cruz et al. 2011; Han et al. 2012). These degradative bacteria have also been found in sewage effluent (Lam et al. 1995), lake water

(Cousins et al. 1996; Jones et al. 1994; Lahti et al. 1997b), and lake sediment (Lahti et al. 1997a; Rapala et al. 1994; U.S. EPA 2015a). Lam et al. (1995) reported that the biotransformation of microcystin-LR followed a first-order decay with a half-life of 0.2 to 3.6 days. In a study done by Jones et al. (1994) with microcystin-LR in different natural surface waters, microcystin-LR persisted for 3 days to 3 weeks; however, more than 95 percent loss occurred within 3 to 4 days. A study by Christoffersen et al. (2002) measured half-lives in the laboratory and in the field of approximately 1 day, driven largely by bacterial aerobic metabolism. These researchers found that approximately 90 percent of the initial amount of microcystin disappeared from the water phase within 5 days, irrespective of the starting concentration. Other researchers (Edwards et al. 2008) have reported half-lives of 4 to 14 days, with longer half-lives associated with a flowing stream and shorter half-lives associated with lakes. Microcystin-LR degradation by *Sphingopyxis* species has been observed with an optimal degradation rate at a pH between 6.5 and 8.5 (Schmidt et al. 2014). Several studies have demonstrated bacterial degradation of microcystin-LR, but other congeners, such as microcystin-LF or -LA, are not significantly degraded (Zastepa 2014; Zastepa et al. 2014). Although microcystin-degrading bacteria might be present, initial degradation could be slow as the bacteria need time to become active (Hyenstrand et al. 2003), and microcystins can accumulate in the water column if these bacteria are not present at the time of a toxic bloom (Schmidt et al. 2014).

Where rivers discharge to the ocean, freshwater cyanobacteria, cyanotoxins, or both can enter the marine environment and this may impact aquatic life in marine environments (Andersen et al. 1993; Miller et al. 2010). Miller et al. (2010) confirmed the transfer of freshwater microcystins to the marine environment. The researchers found that after introducing *Microcystis* cyanobacteria to a saline environment, cyanobacteria can survive for 48 hours before lysing and releasing microcystins. Microcystins concentrations decreased to 29 to 56 percent of the initial concentration after 1 hour in the saline environment, but continued to be detected in the seawater for at least 21 days, based on a detection limit of 0.02 µg/L (Miller et al. 2010). Gibble and Kudela (2014) made additional observations of microcystins at the interface of freshwater and seawater, in the Monterey Bay area, California. In the first year of a 3-year study, microcystin was detected in 15 of 21 fresh-water, estuarine, and marine locations. In the two subsequent years, monitoring focused on four major watersheds that feed into Monterey Bay. The authors observed high concentrations of microcystin in both autumn and spring seasons and concluded that microcystins are likely present throughout the year and transfer to the coastal environment, with the potential to be a persistent issue in the Monterey Bay area. The authors also correlated anthropogenic nutrient loadings with microcystin.

Cylindrospermopsin

Cylindrospermopsin is relatively stable in the dark and at temperatures from 4°C to 50°C for up to 5 weeks (ILS 2000). Cylindrospermopsin is also resistant to changes in pH and remains stable for up to 8 weeks at pH 4, 7, and 10. In the absence of cyanobacterial cell pigments, cylindrospermopsin tends to be relatively stable in sunlight, with a half-life of 11 to 15 days in surface waters (Funari & Testai 2008).

Like microcystin, degradation of cylindrospermopsin increases in the presence of cell pigments such as chlorophyll *a* and phycocyanin, a blue photosynthetic pigment found in cyanobacteria. When exposed to both sunlight and cell pigments, cylindrospermopsin breaks down rapidly, more than 90 percent within 2 to 3 days (Chiswell et al. 1999).

Cylindrospermopsin has been shown to be decomposed by bacteria in laboratory studies; the biodegradation is influenced by the cyanotoxin concentration, temperature and pH. Mohamed and Alamri (2012) reported that cylindrospermopsin was degraded by *Bacillus* bacteria and degradation occurred in 6 days at the highest toxin concentration (300 μg/L) and in 7 or 8 days at lower concentrations (10 and 100 μg/L, respectively). The biodegradation rate was also reported to depend on temperature and pH, with the highest rates occurring in warm waters (25 and 30°C) and neutral to slightly alkaline conditions (pH 7 and 8). Klitzke and Fastner (2012) confirmed the observations of Mohamed and Alamri (2012), noting that a decrease in temperature from 20 to 10°C slowed down degradation by a factor of 10. They also found that degradation slowed significantly under anaerobic conditions, with half-lives of 2.4 days under aerobic conditions and 23.6 days under anaerobic conditions.

3.2.4 Toxicokinetics

Limited data are available regarding the toxicokinetics of microcystins in environmental exposure conditions (U.S. EPA 2015d). Available intestinal data indicate that the organic acid transporter polypeptide (OATp) family transporters facilitate the absorption of microcystins from the intestinal tract into liver, brain, and other tissues, as well as their export out of organs and tissues (Cheng et al. 2005; Fischer et al. 2005; Svoboda et al. 2011). However, bile acids and other physiologically-relevant substrates compete with microcystins for transporter uptake by the liver (Thompson & Pace 1992); reduction or elimination of liver toxicity has been observed during *in vivo* or *in vitro* exposures when microcystin uptake by OATp transporters is limited or inhibited (Hermansky et al. 1990a; Hermansky et al. 1990b; Runnegar et al. 1995; Runnegar & Falconer 1982; Runnegar et al. 1981). Both *in vivo* and *in vitro* studies have shown biliary excretion of microcystins (Falconer et al. 1986; Pace et al. 1991; Robinson et al. 1991), possibly via conjugation with cysteine and glutathione (Kondo et al. 1996). Additional details of microcystin toxicokinetics can be found in U.S. EPA's Drinking Water Health Advisory and HESD for microcystins (U.S. EPA 2015a; U.S. EPA 2015d).

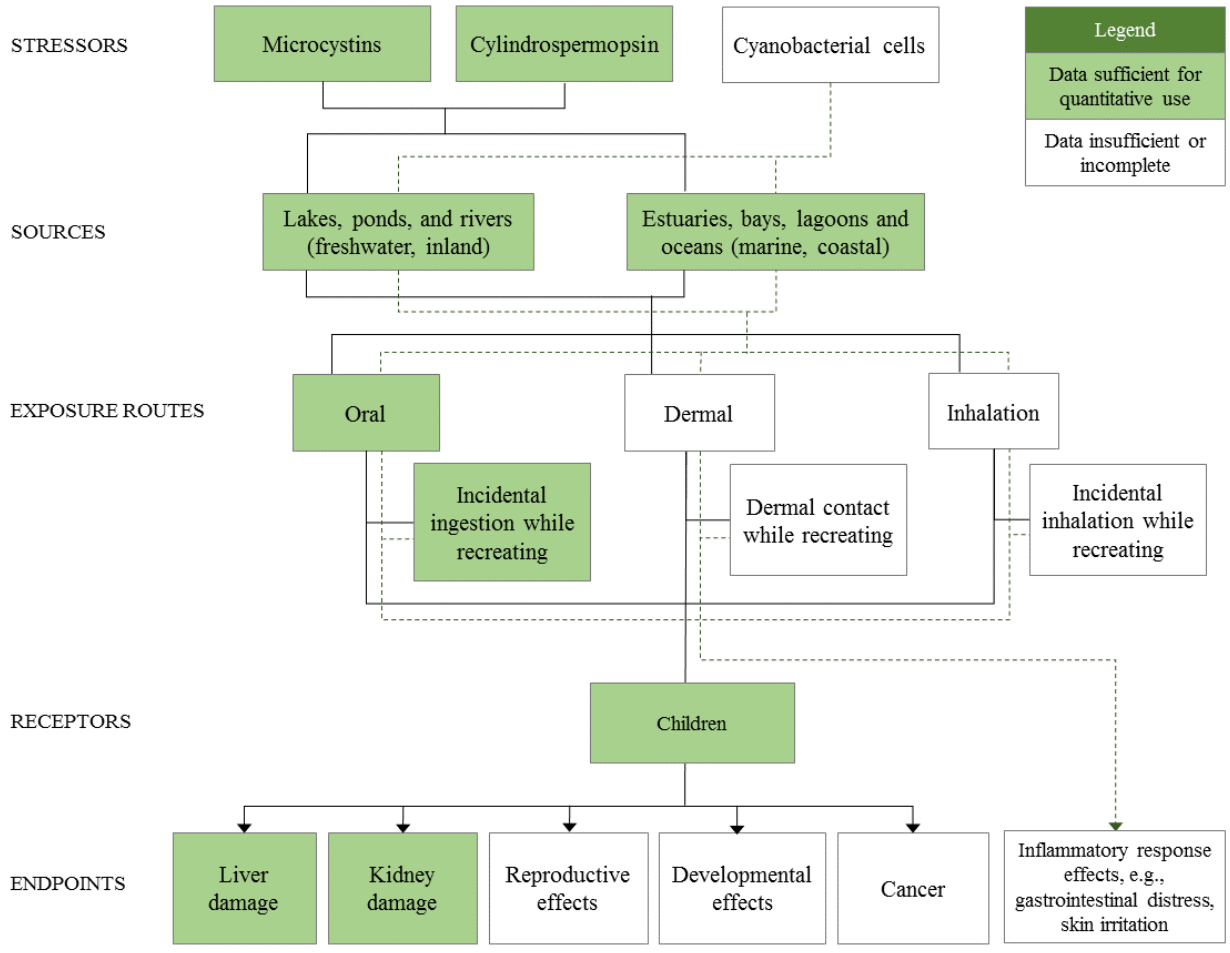
Limited toxicokinetic data for cylindrospermopsin are available, and are derived from mice intraperitoneal studies and *in vivo* studies that do not necessarily reflect environmental exposure conditions (U.S. EPA 2015c). Cylindrospermopsin is absorbed from the GI tract (Humpage & Falconer 2003; Shaw et al. 2001; Shaw et al. 2000) and is distributed primarily to the liver but also to the kidneys and spleen (Norris et al. 2001). The metabolism and toxicity of cylindrospermopsin is mediated by hepatic cytochrome P450 (CYP450) enzymes, and the periacinar region of the liver appears to be the main target of toxicity where cylindrospermopsin and its metabolites bind to proteins (Norris et al. 2001; Runnegar et al. 1995; Shaw et al. 2001; Shaw et al. 2000). Elimination of cylindrospermopsin was continuous over a monitoring period of 24 hours, with a large mean total recovery primarily from urine, and to a smaller extent, feces, after 24 hours (Norris et al. 2001). Additional details of cylindrospermopsin toxicokinetics can be found in EPA's Drinking Water Health Advisory and HESD for cylindrospermopsin (U.S. EPA 2015b; U.S. EPA 2015c).

4.0 PROBLEM FORMULATION

4.1 Conceptual Model

This conceptual model provides useful information to characterize and communicate the potential health risks related to exposure to microcystins and cylindrospermopsin in recreational waters. The sources of cyanotoxins in these waters, the recreational route of exposure for biological receptors of concern, and the potential assessment endpoints (e.g., effects such as kidney and liver toxicity) are depicted in the conceptual diagram below (Figure 4-1).

Figure 4-1. Conceptual Model of Exposure Pathways to the Cyanotoxins, Microcystins and Cylindrospermopsin, and Cyanobacteria in Surface Waters while Recreating



Conceptual Model Diagram for Exposure via Recreational Exposures

The conceptual model is intended to explore potential links of exposure to a contaminant or stressor with the adverse effects and toxicological endpoints important for management goals, including the development of recreational ambient water quality criteria. Boxes that are shaded indicate pathways that EPA considered quantitatively in estimating the advisory level, whereas

the white boxes did not have sufficient data for EPA evaluate quantitatively. The solid lines are for the cyanotoxins and the dotted lines are for the cyanobacterial cells.

Factors Considered in the Conceptual Model for Microcystins and Cylindrospermopsin

Stressors. The stressors are microcystins and cylindrospermopsin concentrations in water. These toxins can be produced by cyanobacteria occurring in freshwater. Once produced, the toxins have the potential to affect downstream waters, including coastal areas. The values recommended in this document could be applied to coastal waters affected by toxins produced by upstream freshwater cyanobacteria. Cyanobacterial cells as direct stressors in recreational surface waters are discussed in Appendix D.

Sources. Cyanobacteria occur naturally in surface waters, such as lakes, ponds, rivers, estuaries, bays, lagoons, and oceans in or surrounding the United States. Some genera of the cyanobacteria, including *Microcystis*, *Cylindrospermopsis*, *Anabaena*, *Planktothrix*, and *Nostoc*, can produce the cyanotoxins microcystins and cylindrospermopsin. Once these toxins are produced, they can be stable in the environment for weeks (Funari & Testai 2008; Zastepa et al. 2014).

Routes of exposure. Exposure to cyanotoxins from recreational water sources can occur via oral exposure (incidental ingestion while recreating); dermal exposure (contact of exposed parts of the body with water containing cyanotoxins during recreational activities such as swimming, wading, surfing); and inhalation exposure to contaminated aerosols (while recreating). The route of exposure considered quantitatively is oral exposure to microcystin and cylindrospermopsin via incidental ingestion while swimming. Dermal exposure happens during swimming; however, significant dermal absorption of microcystins and cylindrospermopsin is not expected due to the large size and charged nature of these molecules (Butler et al. 2012; U.S. EPA 2004; U.S. EPA 2007). EPA estimated that ingestion from inhalation is likely negligible compared to incidental ingestion while recreating (see section 7.5.1.1). Routes of exposure other than ingestion of drinking water are taken into account by the application of a relative source contribution value (U.S. EPA 2000a). Routes of exposure other than incidental oral ingestion while swimming are discussed further in the Effects Characterization, section 7.5.1.

Receptors. Anyone who recreates in a water body where cyanotoxins are present could be exposed to cyanotoxins through ingestion, dermal contact, and inhalation of aerosols while recreating in contaminated surface waters. Childhood is considered a vulnerable lifestage due to children's potential increased exposure while recreating. Recreating children can be at greater risk from exposure to microcystins or cylindrospermopsin because they have smaller body mass compared to adults, they spend more time in contact with the water compared to adults, and they incidentally ingest more water than adults while recreating. Thus, EPA is specifically considering the recreational exposures children experience in this assessment. EPA evaluates and discusses differences between lifestages in section 7.4 of the Effects Characterization. While there are many examples in the literature and reports of animal poisonings and death from exposure to cyanotoxins, values protective of animals such as dogs and livestock are not generated in this document. However, section 7.6 discusses some animal specific issues, including a summary of guidelines several states have developed for animals.

Endpoints. Available microcystin toxicity data indicate that the primary target organ for microcystins is the liver as described in EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* (U.S. EPA 2015d). Available cylindrospermopsin toxicity

data are described in EPA's *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA 2015c). For cylindrospermopsin, EPA selected kidney effects as the endpoint on which to base the measure of effect. Clinical, epidemiological, and outbreak study results (see Appendix D) suggest a link between an increase in adverse inflammatory symptoms among recreators and elevated cyanobacterial cell densities. However, there is considerable uncertainty and variability associated with the epidemiological results, which did not identify consistent effects at similar cyanobacterial densities. Specifically, significant associations occur across a wide range of cell densities; associations vary with different specific health endpoints or combined symptom categories; and differences in cyanobacterial community composition are largely uncharacterized. These endpoints are not considered quantitatively in this assessment, but potential health effects are described in the Effects Characterization section 7.1 along with a discussion of the uncertainties related to the data for cyanobacterial cells.

4.2 Analysis Plan

EPA's 2000 *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000 Human Health Methodology) outlines EPA's process for deriving Ambient Water Quality Criteria (AWQC) and guides the development of these recreational criteria and swimming advisories (U.S. EPA 2000a).

The 2000 Human Health Methodology includes identifying the population subgroup that should be protected, evaluation of cancer and non-cancer endpoints, measures of effect, measures of exposure, relative source contribution (RSC), and evaluation of bioaccumulation. In this analysis plan, EPA: (1) describes the RfD previously derived for microcystin and cylindrospermopsin (measure of effect); (2) describes the calculation for the recreational criteria; (3) discusses incidental ingestion exposure in terms of volume ingested, duration of exposure, and body weight (measure of exposure) described in EPA's *Exposure Factors Handbook* and; (4) discusses the RSC. These criteria focus on human exposure as a result of primary contact recreation activities such as swimming where immersion and incidental ingestion of ambient water are likely.

EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* and *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA 2015c; U.S. EPA 2015d) provide the health effects basis for the development of the Drinking Water Health Advisories for microcystins and cylindrospermopsin (U.S. EPA 2015a; U.S. EPA 2015b), including the science-based decisions providing the basis for estimating the point of departure. To develop the HESDs for microcystins and cylindrospermopsin, EPA assembled available information on toxicokinetics, acute, short-term, subchronic and chronic toxicity along with developmental and reproductive toxicity, neurotoxicity, immunotoxicity, genotoxicity and cancer in humans and animals. For detailed descriptions of the literature search strategies, see the HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c; U.S. EPA 2015d). This document was subject to rigorous internal and external peer review before it was finalized in 2015. The information evaluated for these documents also supports the development of the recreational criteria and swimming advisories for microcystins and cylindrospermopsin, which evaluate exposure via recreational water ingestion. EPA conducted supplemental literature searches in September 2015 to capture new references, including effects related to recreational exposure to cells. For detailed information search terms, see Appendix C.

4.2.1 Approach for Recreational AWQC Derivation

The Recreational AWQC for microcystins and cylindrospermopsin are calculated as described in the 2000 Human Health Methodology and presented in the equation below:

$$\text{Recreational AWQC } (\mu g/L) = \frac{RfD \times RSC \times BW}{IR}$$

Where:

RfD	=	Reference dose (μg/kilograms [kg] body weight [bw]/day [d])
RSC	=	Relative source contribution (RSC is discussed in section 4.2.5).
BW	=	Mean body weight (kg)
IR	=	Ingestion rate (L/d) at approximately the 90th percentile (discussed in section 4.2.3)

4.2.1.1 Magnitude, Duration and Frequency

EPA recommends that recreational criteria consist of a magnitude, duration, and frequency. Magnitude is the numeric expression of the maximum amount of the contaminant that may be present in a waterbody that supports the designated use. Duration is the period of time over which the magnitude is calculated. Frequency of excursion describes the number of times the pollutant may be present above the magnitude over the specified time period (duration). A criterion is derived such that the combination of magnitude, duration, and frequency protect the designated use (e.g., primary contact recreation). For microcystins and cylindrospermopsin, the magnitude of the criteria is based on the data used to derive the toxicity (in this case the RfDs for microcystins and cylindrospermopsin) values developed to support the Drinking Water Health Advisories (U.S. EPA 2015a,b). The duration and frequency components of the criteria are consistent with the approach discussed in previous recreational criteria, including the application of the recommended magnitudes using different durations for beach management and waterbody assessment.

4.2.2 Measures of Effect

A reference dose or RfD is an estimate (with uncertainties spanning perhaps an order of magnitude) of the daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. EPA's HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c,d), provide the health effects basis for development of the reference dose or RfD, including the science-based decisions (i.e., selection of the critical study and endpoints) providing the basis for estimating the point of departure and application of uncertainty factors. EPA uses the RfD values for oral exposure previously peer reviewed and documented in the HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c; U.S. EPA 2015d) in derivation of the recreational criteria and swimming advisories. Dermal exposure happens during swimming; however, significant dermal absorption of microcystins and cylindrospermopsin is not expected due to the large size and charged nature of these molecules (Butler et al. 2012; U.S. EPA 2004; U.S. EPA 2007). Because available data are

not sufficient, EPA is not quantifying effects resulting from dermal exposure to cyanotoxins. See section 7.5.1.2 for a characterization of dermal exposure to these cyanotoxins.

Inhalation exposure occurs during swimming; however, data are not sufficient to quantify health effects resulting from inhalation exposure to cyanotoxins at this time. See section 7.5.1.1 for a characterization of potential effects from inhalation exposure.

Dermal exposure to cyanobacterial cells can also result in adverse health effects, such skin rashes, eye irritation, and ear irritation. Because adequate data are not available, EPA is not quantifying effects resulting from exposure to cells at this time. Available epidemiological study results do not provide consistent associations between cell densities and the inflammatory health endpoints. Some of the studies have been limited in size, which could affect the ability to detect an association if one exists. Differences in the cyanobacterial communities present at the study sites may have affected the detection of associations. Characterization of confounders, such as the presence of pathogens, was not consistent among the studies. See section 7.1.1 for a characterization of potential effects from recreational exposure to cyanobacterial cells.

4.2.3 Measures of Exposure

The exposure parameters selected for use in calculating recreational criteria and swimming advisories for microcystins and cylindrospermopsin include an ingestion rate (volume of surface water incidentally ingested per day) and body weight (kg). Both body weight and incidental ingestion while recreating are parameters that vary with age. The key study and other data supporting these exposure factors are described in the sections that follow.

All recreational exposure studies that included both children and adults found that age could influence incidental ingestion exposure while recreating. More specifically, children tend to ingest more water and spend more time in the water compared to adults (Dufour et al. 2006; Schets et al. 2011; U.S. EPA 1997). EPA's *Exposure Factors Handbook* (U.S. EPA 2011) provides recommended values for body weights and incidental ingestion volumes and rates for children and adults on an event basis. The *Handbook* recommends using the 97th percentile ingestion rate for children and the maximum reported value for adults because the dataset is limited (U.S. EPA 2011).

EPA's *Exposure Factors Handbook* (2011) edition and (1997) edition provided values for time spent swimming per month and time spent in a pool/spa per day, respectively. EPA (2011) compiled mean and 95th percentile swimming durations for different children's age groups in minutes/month (e.g., mean swimming duration value for children 1 to < 2 years was 105 minutes/month, for children 3 to < 6 years was 137 minutes/month, and for children 6 to 11 years was 151 minutes/month). EPA needed a duration parameter expressed as time exposed per day to calculate a daily ingestion rate. Converting the monthly values to daily durations (e.g., dividing the monthly value by 30 days per month) resulted in very short daily exposures that do not seem reasonable given the other duration estimates available. Therefore, EPA used the duration of recreational event per day reported in U.S. EPA (1997) of 2.7 hours per day for children 5 to 11 years old.

4.2.3.1 Incidental Ingestion

Primary Contact Exposure Scenario

EPA selected incidental ingestion during primary contact activities such as swimming for the criteria derivation because data suggest that incidental ingestion can be considered the highest potential exposure pathway for cyanotoxins while recreating. In a combined analysis of 2,705 individuals recreating in the Chicago Area Waterway System and 662 individuals recreating at a public outdoor swimming pool, Dorevitch et al. (2011) studied the volume of water ingested during a range of recreational activities. Study subjects took part in one of the following activities: canoeing, fishing, kayaking, motor boating, rowing, wading/splashing, head immersion (i.e., immersed one's head three times over a 10-minute interval), or swimming. At the end of their exposure, participants self-reported whether they ingested water, and how much, during their recreational experience. The results indicate that the odds of ingesting a teaspoon or more of water are significantly higher among swimmers than among those who just immersed their head in a swimming pool or those who participated in the other, more limited contact activities on surface waters. More specifically, rowing, motor boating, fishing, wading/splashing, and non-capsizing kayaking and canoeing were found to be low-ingestion activities, resulting in 95th percentile ingestion volumes between 0.01 and 0.012 L/hr. The study authors considered those who capsized during canoeing or kayaking a "middle ingestion category," with a 95th percentile ingestion volume of about 0.017 to 0.02 L/hr. Swimmers were the highest ingestion category, with a 95th percentile ingestion volume of approximately 0.035 L/hr. Evaluations of inhalation (see section 7.5.1.1) and dermal (see section 7.5.1.2) exposures suggest that those two routes are minor compared to the oral exposure route. Thus, EPA determined that using a swimmer scenario for exposure as the basis for the criteria is protective of these other aquatic activities.

Incidental Ingestion per Day

To calculate the recreational incidental ingestion rate in units of volume per day, EPA combined a distribution from EPA's (2011) *Exposure Factors Handbook* on incidental ingestion volumes (volume per event normalized to volume per hour) and a distribution of exposure durations (hours per day) from EPA's (1997) *Exposure Factors Handbook*. The recommended 97th percentile incidental ingestion volume for children combined with the mean exposure duration represented the 90th percentile of this combined distribution to represent incidental ingestion per day. These data are discussed in the following sections.

Ingestion Volume Studies

EPA's *Exposure Factors Handbook* (2011) cites Dufour et al. (2006) as the basis for its default recreational ingestion values. Dufour et al. (2006) measured the incidental ingestion of water while participants were swimming in a pool and found that children under the age of 18 years ingested higher volumes of water while swimming than adults and that males ingested more than females. This small-scale pilot study (n = 53) used cyanuric acid as an indicator of amount of pool water ingested while swimming in an outdoor pool. Participants were instructed

to stay in the pool and actively swim for at least 45 minutes. Pool-water samples were collected before the start of swimming activities, and participants' urine was collected for 24 hours after the swimming event ended; pool-water and urine samples were analyzed for cyanuric acid. The combined study population had a mean incidental ingestion volume of 0.019 L per swimming event. Because sample size for the Dufour et al. (2006) study was small (i.e., 41 children and 12 adults), the authors reported results for children under the age of 18 years and adults. In addition, children younger than 6 years were not included in the study design. One study by Schets et al. (2011) reported surveyed parents' estimates of incidental ingestion for children younger than 6 years old. The Schets et al. (2011) reported ingestion values for children ages 0 to < 15 years were similar to the Dufour et al. (2006) findings; see section 7.3 for more detail.

The values presented in EPA's *Exposure Factors Handbook* (2011) adjusted the Dufour et al. (2006) data from a per event (e.g., 45 minutes) basis to an hourly ingestion rate. The distribution of Dufour et al. (2006) measured incidental ingestion rates are graphically presented in Figure 4-2. Based on these data, the *Exposure Factors Handbook* recommended assessments use a 97th percentile (0.12 L/hr) for children and a maximum value (0.071 L/hr) for adults as "upper percentile" values due to the limited sample size of the Dufour et al. (2006) study. Several other studies (Dufour et al. 2006; Schets et al. 2011; Suppes et al. 2014; U.S. EPA 2000a) characterizing incidental ingestion while swimming are available and described in the effects characterization section (section 7.3). These other studies reported similar results to Dufour et al. (2006).

Duration of Recreational Exposure

Duration of recreational exposure quantifies the length of time people might be exposed to cyanotoxins during their primary contact recreational use of surface waters contaminated with cyanotoxins. Duration of recreational exposure is needed to convert recreational ingestion rates in units of volume per hour to an amount incidentally ingested per day, which is the exposure parameter needed for the recreational AWQC derivation.

EPA's *Exposure Factors Handbook* (1997) lists, for different age groups, time spent per 24 hours in an outdoor spa or pool, which is interpreted for purposes of this calculation as time spent in direct contact with water, for example, swimming. The data are based on analysis of the National Human Activity Pattern Survey by Tsang and Klepeis (1996). Figure 4-3 compares the recreational duration data for different age groups and shows that recreators ages 5 to 11 years tend to spend more time in the water than other child age groups and adults, although the 90th percentile values are similar. A duration was not provided for children younger than 1 year. EPA evaluated both the mean duration for the various age groups and available exposure parameters for children younger than 6 years old; see section 7.4.

Other data show a similar trend of longer recreational durations for children. Schets et al. (2011) investigated swimming durations in freshwater, marine water, and pools. They surveyed 8,000 adults, 1,924 of whom also provided estimates for their eldest child (< 15 years of age) and found that children spend, on average, 25 minutes longer swimming in freshwaters compared to adults. The mean duration of swimming events for children ages 0–14 years in freshwater and marine water were 79 minutes (1.3 hours, 95 percent CI: 12–270 minutes) and 65 minutes (1.1 hours, 95 percent CI: 8–240 minutes), respectively. Adult averages were all less than 1 hour.

Figure 4-2. Incidental Ingestion Rates Measured for Adults and Children (Dufour et al. 2006)

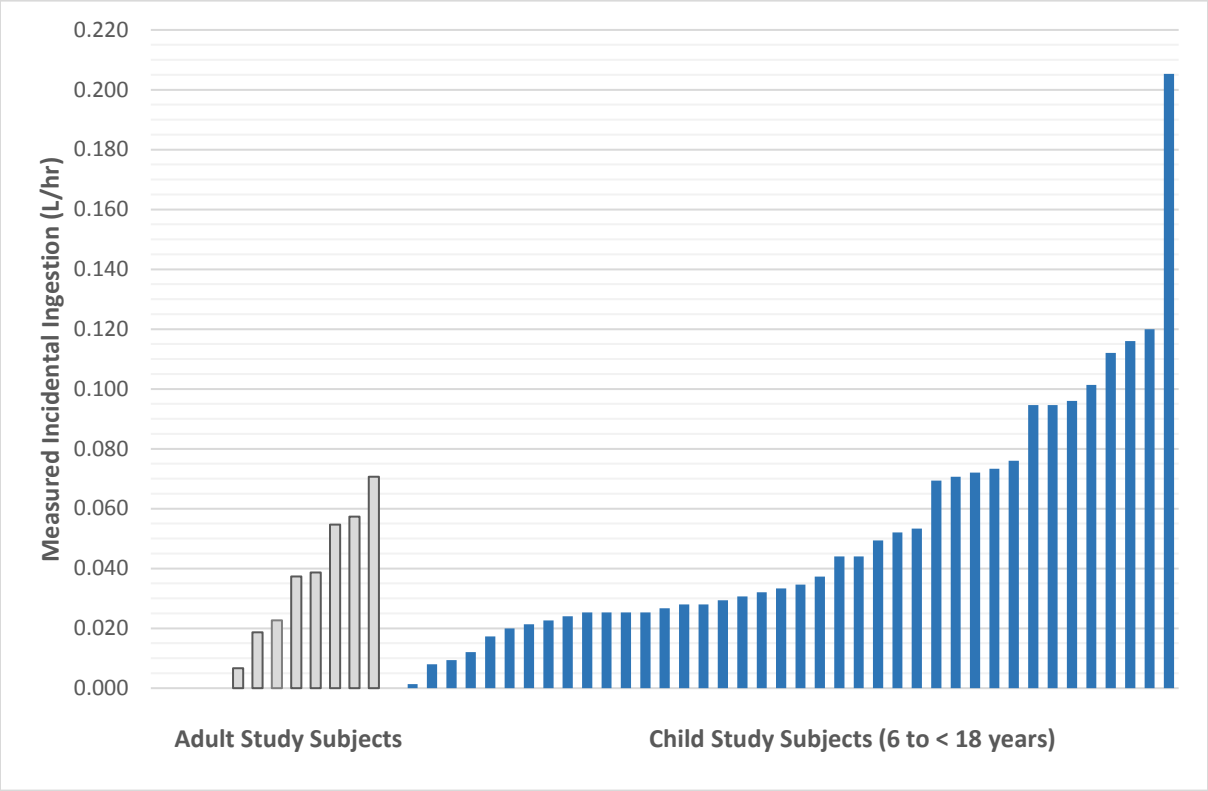
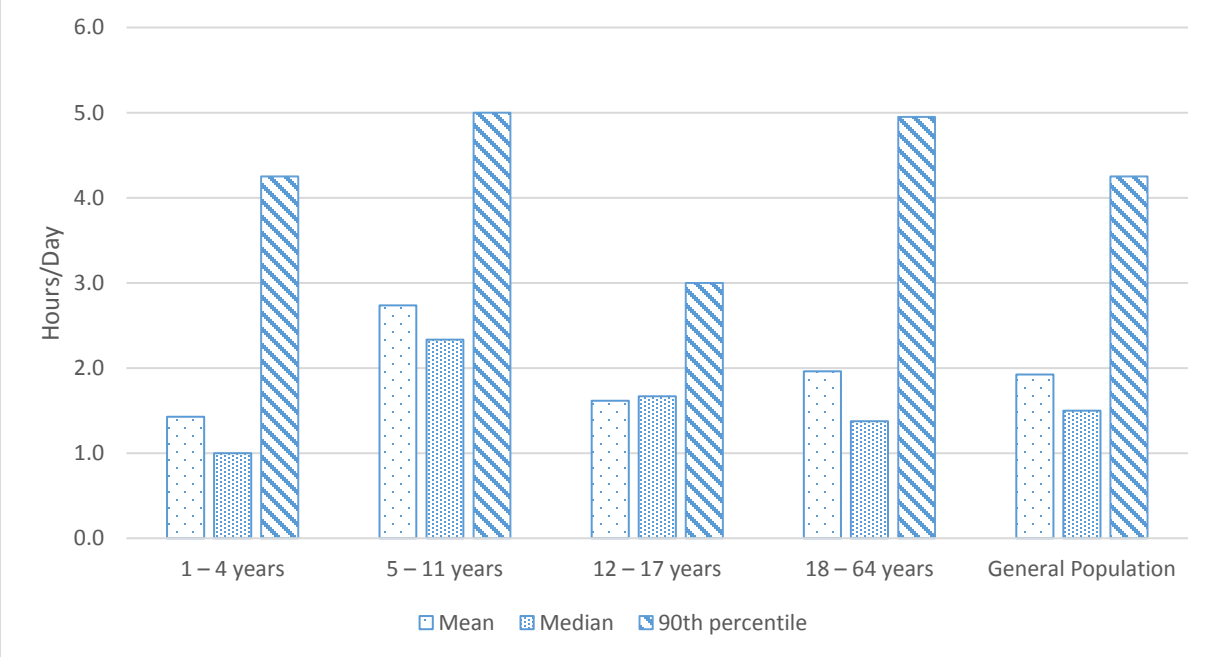


Figure 4-3. Direct Contact Recreational Exposure Duration by Age Group, Based on Table 15-119 in U.S. EPA (1997)



Additional duration estimates of children's pool swimming have been identified by EPA's Office of Pesticide Programs for use in its Swimmers Exposure Assessment Model (SWIMODEL) for estimating chemical exposures during pool swimming, including direct contact for competitive swimmers (U.S. EPA 2003). EPA's SWIMODEL considers short-term exposure (using a high-end estimate of exposure time per event in order to represent a maximum, one-time exposure) and intermediate/long-term exposure (using a shorter event duration to represent an average of maximum and minimum exposures overtime). Among competitive children swimmers, the longest short-term exposure duration used by the SWIMODEL is 2 hours/day for children ages 11–15 years (U.S. EPA 2003). Competitive swimming practice durations, however, are less relevant for recreational scenarios in lakes and rivers than for exposure in a pool. EPA's *Exposure Factors Handbook* (1997) lists the mean exposure duration for children 5 to 11 years as 2.7 hours/day, which is longer than the maximum value for children 11 to 15 years used in the SWIMODEL.

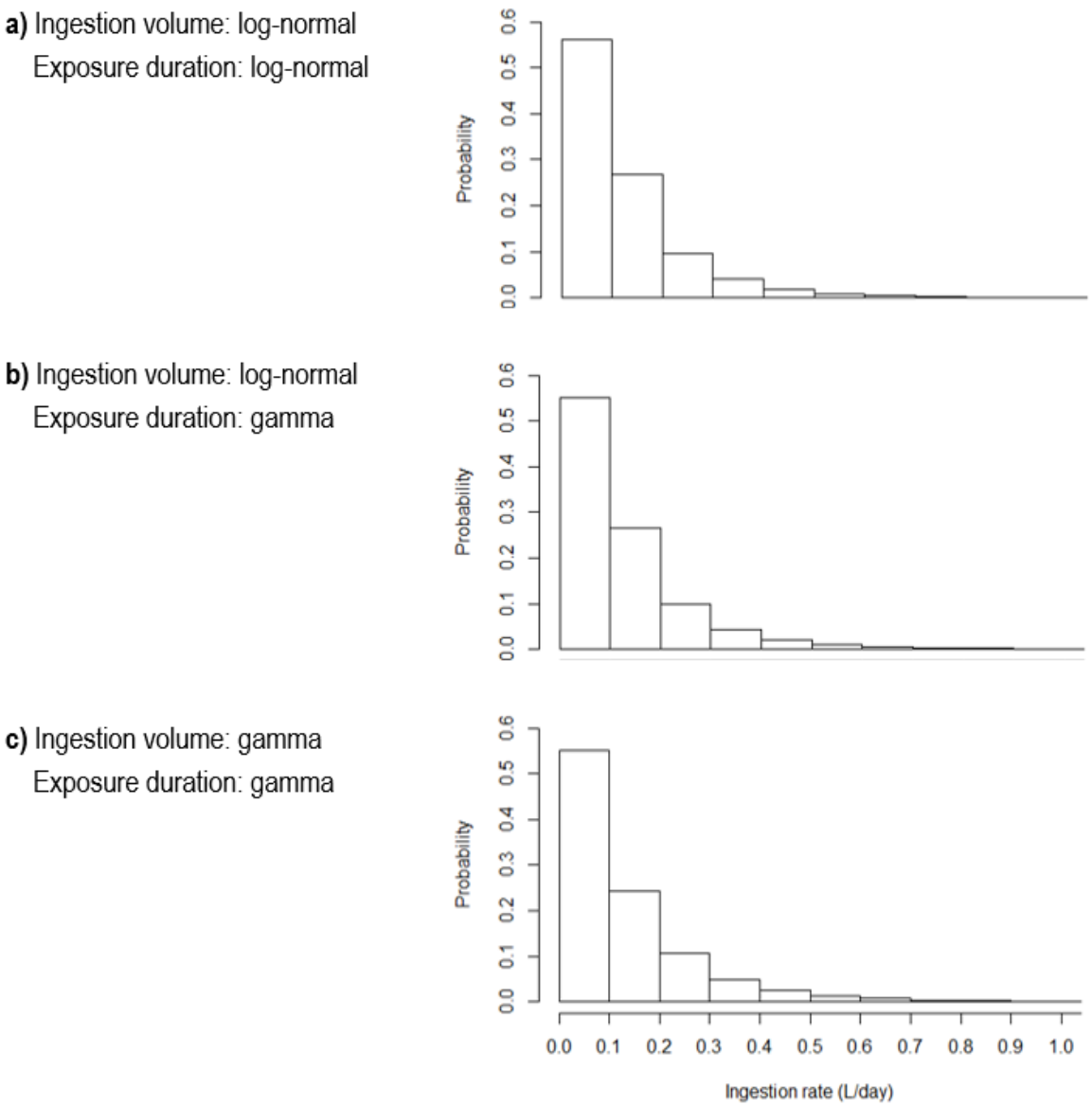
Determination of Incidental Ingestion per Day

The incidental ingestion volume per day EPA used to calculate the recreational criteria or swimming advisory is the product of the 97th percentile children's incidental ingestion rate (0.12 L/hr) and mean exposure duration (2.74 hr/day) for children ages 5 to 11 years. EPA evaluated the effect these multiple parameters had on the level of protection by analyzing the combined distributions of ingestion volume per hour and duration of recreational exposure. EPA compiled the published statistical parameters (i.e., mean, standard deviation, and minimum and maximum data values) and evaluated the resulting distributions for both parameters compared to a normal, log-normal, and gamma function. For both parameters, the log-normal or gamma functions better described the distributions. Log-normal and gamma functions are strictly positive distributions and reflect the apparent skewness in the data. Describing a distribution with a normal function can result in negative values that are not representative. In the analysis, both distributions were limited to their respective minimum and maximum data values. Table 4-1 shows the statistics of the combined distributions: (a) a lognormal distribution for both parameters, (b) a lognormal distribution for the ingestion rate and a gamma distribution for the exposure duration, and (c) a gamma distribution for both parameters. The combinations of the two distributions assuming log-normal, gamma, or both, are shown in Figure 4-4 as hybrid distributions. For all three combined distributions using combinations of log-normal and/or gamma functions, the incidental ingestion rate per day (0.33 L/d) represents approximately the 90th percentile of the hybrid distributions (range 92nd to 94th percentile). Additional details including the methodology for this analysis are provided in Appendix E.

Table 4-1. Summary Statistics of Combined Ingestion Volume and Exposure Duration Distributions

Combined Distribution	Ingestion Volume (L/hr) Distribution	Exposure Duration (hr/d) Distribution	Summary Statistics for Ingestion Rate (L/d)				
			Minimum	Median	Mean	Maximum	Percentile Associated with 0.33 L/d
a	Log-normal	Log-normal	0.0023	0.0873	0.12	1.47	94
b	Log-normal	Gamma	0.0018	0.0888	0.13	1.46	93
c	Gamma	Gamma	0.0000	0.0871	0.13	1.40	92

Figure 4-4. Hybrid Distributions for Incidental Ingestion per Day (L/d)



4.2.3.2 Body Weight

Table 8-1 in EPA's *Exposure Factors Handbook* (U.S. EPA 2011) reported recommended and other body weight statistics based on the National Health and Nutrition Examination Survey. A range of age groups is included. Mean body weight for children aged 6 to < 11 years was 31.8 kg. EPA selected this body weight because it reflected the age group with higher ingestion volumes (U.S. EPA 2011; Evans et al. 2006) and exposure duration (U.S. EPA 1997). A discussion of younger children's exposure factors can be found in section 7.4.2.

4.2.4 Relative Source Contribution

EPA's 2000 Human Health Methodology (2000a) outlines EPA's process for deriving AWQC and guides the development of these recreational criteria. The 2000 Human Health Methodology recommends the application of a RSC in the AWQC derivation to ensure that an individual's total exposure from all routes of exposure to a contaminant does not exceed the RfD. EPA considered the 2000 Human Health Methodology's Exposure Decision Tree Approach to determine the RSC used in deriving the recreational values for microcystins and cylindrospermopsin (Figure 4-1 in the 2000 Human Health Methodology document).

The RSC component of the AWQC calculation allows a percentage of the RfDs exposure to be attributed to the consumption of ambient water and fish and shellfish from inland and nearshore waters when there are other potential exposure sources. The RSC describes the portion of the RfD available for AWQC-related sources (USEPA 2000a); the remainder of the RfD is allocated to other sources of the pollutant. The rationale for this approach is that for pollutants exhibiting threshold effects, the objective of the AWQC is to ensure that an individual's total exposure from all sources does not exceed that threshold level. Exposures outside the RSC include, but are not limited to, exposure to a particular pollutant from fish and shellfish consumption, non-fish food consumption (e.g., fruits, vegetables, grains, meats, poultry, dietary supplements), dermal exposure, and respiratory exposure.

Cyanotoxins are produced by cyanobacteria. As discussed previously, certain environmental factors can lead to rapid growth of cyanobacteria in ambient water. Because environmental factors are not always favorable for cyanobacterial growth, blooms and the production of cyanotoxins are episodic in nature; therefore, determination of background levels is not relevant for cyanotoxins in determining the RSC.

EPA determined that an RSC of 80 percent, as recommended in EPA's 2000 Human Health Methodology, is appropriate for microcystins and cylindrospermopsin. The use of this RSC means that 80 percent of recreators' exposure to cyanotoxins is from incidental ingestion of ambient water during recreational activities. The application of an RSC 80 percent takes into account the uncertainty associated with effects from dermal and inhalation exposures, exposure to contaminated fish and shellfish or drinking water (i.e., 20 percent is set aside for these other exposure routes). An RSC of 80 percent represents the ceiling for setting an RSC, and provides a margin of safety for individuals, given currently available data on exposure to different sources and via other routes.

5.0 EFFECTS ASSESSMENT

The health effects studies summarized below for microcystin and cylindrospermopsin are described in detail in EPA's HESDs and Drinking Water Health Advisories for these two cyanotoxins (U.S. EPA 2015a,b,c,d).

5.1 Hazard Identification

5.1.1 Noncancer Health Effects

5.1.1.1 Animal Toxicity Studies

Microcystins

Studies in laboratory animals demonstrate liver, kidney, and reproductive effects following short-term and subchronic oral exposures to microcystin-LR. Studies evaluating the chronic toxicity of microcystins have not shown clinical signs of toxicity and are limited by study design and by the lack of quantitative data. Observed effects in animals exposed orally or via intraperitoneal (i.p.) to microcystin-LR include liver, reproductive, developmental, kidney, and GI effects.

The preponderance of animal toxicity data on the noncancer effects of microcystins is restricted to the microcystin-LR congener. Studies evaluating the chronic toxicity of microcystins have not shown clinical signs of toxicity and are limited by study design and by the lack of quantitative data. Available data on the RR, YR, and LA congeners did not provide dose-response information sufficient for quantification. EPA is using data on effects of microcystin-LR to represent other microcystin congeners (U.S. EPA 2015d).

For details see the *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* (U.S. EPA 2015d).

Cylindrospermopsin

Based on oral and i.p. studies in mice treated with purified cylindrospermopsin or extracts of C. raciborskii cells, the liver and kidneys appear to be the primary target organs for cylindrospermopsin toxicity.

No oral reproductive or developmental studies are available for cylindrospermopsin. Developmental toxicity studies following i.p. administration of cylindrospermopsin provide some evidence for maternal toxicity and decreased postnatal pup survival and body weight (Chernoff et al. 2011; Rogers et al. 2007).

For details, see EPA's *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA 2015c).

5.1.1.2 Human Studies

Microcystins

Limited human studies examining microcystin effects on humans are available; however, no dose-response data are available from ambient exposures to microcystins. The scant human data on the oral toxicity of microcystin-LR are limited by the potential co-exposure to other pathogens, cyanotoxins, and microorganisms; by the lack of quantitative information; and by the failure to control for confounding factors. Available human studies evidence is supportive of the liver as a target organ for toxicity (Carmichael 2001; Falconer et al. 1983; Giannuzzi et al. 2011; Hilborn et al. 2013; Jochimsen et al. 1998; Li et al. 2011b).

More detailed information on the human health effects of microcystins based on epidemiological studies related to drinking water outbreaks, clinical studies, and cases studies are discussed in the *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* (U.S. EPA 2015d). Of the epidemiological studies EPA identified, three studies evaluated human health effects associated with recreational exposures to cyanobacteria and microcystins. These studies are also summarized in the microcystins HESD and are summarized below.

- Backer et al. (2008) conducted an epidemiological study in a small lake in the United States and compared microcystin concentrations in blood and reported symptoms in people recreating in a lake with a *M. aeruginosa* bloom to those of people recreating in a nearby bloom-free lake. Low levels of total microcystins (detection limit = 0.08 ng/m^3) were detected in air samples collected above a lake bloom. Phytoplankton counts ranged from 175,000 to 688,000 cells per mL with > 95 percent of those cells being cyanobacteria. Cell densities of potentially toxigenic cyanobacteria ranged from approximately 54,000 to 144,000 cells/mL. Although a visible bloom was present and contained cyanobacterial species capable of producing microcystin, microcystin concentrations in water during the study ranged from 2 to 5 μg/L. Recreational users of the lake at the time of the bloom had no detectable microcystin in their blood and did not report an increase in GI, dermal, respiratory, or neurological symptoms after spending time on the lake. Adenoviruses (level of detection [LOD] = 1,250 gene copy equivalents) and enteroviruses (LOD = 200 plaque forming units/10 L) were not detected in any water sample. This study was limited in the number of participants and included a limited number of exposure days in the analysis. Given a small number of recreators exposed to low levels of microcystin over the course of 3 study days, the lack of significant associations is not surprising.
- In a similar study conducted by this same author at three lakes in California, microcystin concentrations from personal air samples ranged from the limit of detection (0.1 ng/m^3) to 0.4 ng/m^3, and extracellular microcystin concentrations in water ranged from < 2 μg/L to > 10 μg/L (Backer et al. 2010). No statistically significant differences were noted in the frequency of reported GI, dermal, or respiratory symptoms between participants immediately after they engaged in direct- or indirect-contact recreational activities in the lake with a cyanobacterial bloom and those in a lake without a cyanobacterial bloom. The study design characterized the potential inhalation of aerosolized microcystin among people who recreated at the lakes and included blood assays for microcystin among the study participants. Adenoviruses or enteroviruses were not detected at the study

locations. This study contained a limited number of participants over the course of 3 exposure days.

- Lévesque et al. (2014) conducted a prospective study of residents living in proximity to three lakes in Canada affected by cyanobacteria to investigate the relationship between recreational exposure, specifying full contact and limited contact with lake water and the incidence of GI, dermal, respiratory, and other (e.g., ear pain, muscle pain) symptoms. Full contact included swimming, waterskiing, windsurfing, use of watercraft involving launching, accidental falls, and similar activities, and limited contact included fishing, use of watercraft not involving launching, and other activities. No associations were observed between any symptoms and recreational exposures to microcystins. The maximum microcystin concentrations for which recreational-related GI symptoms were reported was 7.65 μg/L. The authors did observe a relationship between cyanobacterial cell counts and gastrointestinal illness with a significant association above 20,000 cells/mL.
- Additional outbreak and case reports document health effects following exposure to cyanotoxins. In a case report, acute intoxication with microcystin-producing cyanobacteria blooms in recreational water was reported in Argentina in 2007 (Giannuzzi et al. 2011). A single person was immersed in a *Microcystis* bloom containing 33,680 and 35,740 cells/mL. A level of 48.6 μg/L of microcystin-LR concentrations was detected in water samples associated with the bloom. After 4 hours of exposure, the patient exhibited fever, nausea, and abdominal pain, and 3 days later, presented dyspnea and respiratory distress and was diagnosed with an atypical pneumonia. One week after the exposure, the patient developed a hepatotoxicosis with a significant increase of alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase. The patient completely recovered within 20 days.
- Dziuban et al. (2006) and Hilborn et al. (2014) reported nine outbreaks associated with recreational exposure to HABs in which microcystins were detected, one in 2004 and eight in 2009 and 2010. In the one outbreak in which microcystin was measured at 20.8 μg/L and other cyanotoxins were either not detected or measured, 9 cases reported symptoms, which included abdominal cramps (3 cases), diarrhea (3), nausea (3) vomiting (2), fever (2), headache (2), rash (8), eye irritation (1), earache (1), neurologic symptoms (2), tingling (2), confusion (1), and respiratory symptoms (1) (Hilborn et al. 2014). Cyanobacterial cells were present. The results reported from the outbreaks should not be interpreted as cause and effect. Rather, the stressors and health endpoints discussed can be considered a co-occurrence due to the nature of the data collated in the outbreak reports.

Cylindrospermopsin

No epidemiological studies were found for recreational exposure to cylindrospermopsin.

Hilborn et al. (2014) reported two outbreaks associated with recreational exposure to HABs in which cylindrospermopsin was detected between 2009 and 2010. Cyanobacteria, microcystins, and other cyanotoxins, however, also were detected in these two outbreaks. As mentioned above, the results reported from the outbreaks should not be interpreted as cause and

effect, only that two or more parameters were demonstrated to co-occur spatially and/or temporally.

5.1.1.3 Noncancer Mode of Action

Microcystins

Mechanistic studies have shown the importance of membrane transporters for systemic uptake and tissue distribution of microcystin by all exposure routes (Feurstein et al. 2010; Fischer et al. 2005). The importance of the membrane transporters to tissue access is demonstrated when a reduction in, or lack of, liver damage happens following OATp inhibition (Hermansky et al. 1990a; Hermansky et al. 1990b; Thompson & Pace 1992).

The uptake of microcystins causes protein phosphatase inhibition and a loss of coordination between kinase phosphorylation and phosphatase dephosphorylation, which results in the destabilization of the cytoskeleton. This event initiates altered cell function followed by cellular apoptosis and necrosis (Barford et al. 1998). Both cellular kinases and phosphatases keep the balance between phosphorylation and dephosphorylation of key cellular proteins controlling metabolic processes, gene regulation, cell cycle control, transport and secretory processes, organization of the cytoskeleton, and cell adhesion. Each of the microcystin congeners evaluated (LR, LA, and LL) interacts with catalytic subunits of protein phosphatases PP1 and PP2A, inhibiting their functions (Craig et al. 1996).

As a consequence of the microcystin-induced changes in cytoskeleton, increases in apoptosis and reactive oxygen species (ROS) occur. In both *in vitro* and *in vivo* studies, cellular pro-apoptotic Bax and Bid proteins increased while anti-apoptotic Bcl-2 decreased (Fu et al. 2005; Huang et al. 2011; Li et al. 2011a; Takumi et al. 2010; Weng et al. 2007; Xing et al. 2008). Mitochondrial membrane potential and permeability transition pore changes (Ding & Nam Ong 2003; Zhou et al. 2012) lead to membrane loss of cytochrome c, a biomarker for apoptotic events. Wei et al. (2008) identified a time-dependent increase in ROS production and lipid peroxidation in mice after exposure to microcystin-LR. After receiving a 55 μg/kg of body weight i.p. injection of microcystin-LR, the levels of hepatic ROS increased rapidly within 0.5 hours and continued to accumulate for up to 12 hours in a time-dependent manner.

Cylindrospermopsin

Despite the number of studies that have been published, the mechanisms for liver and kidney toxicity by cylindrospermopsin are not completely characterized.

The occurrence of toxicity in the liver suggests a protein-synthesis inhibition mechanism of action for cylindrospermopsin. *In vitro* and *in vivo* studies have been conducted to demonstrate the ability of cylindrospermopsin to inhibit hepatic protein synthesis, which could impact mouse urinary protein production leading to decreased urinary excretion of these proteins (Froscio et al. 2009; Froscio et al. 2008; Terao et al. 1994). Available evidence indicates that protein synthesis inhibition is not decreased by broad-spectrum CYP450 inhibitors, but they do reduce cytotoxicity (Bazin et al. 2010; Froscio et al. 2003). Hepatotoxicity appears to be CYP450-dependent, which indicates a possible involvement of oxidized and/or fragmented metabolites and mechanisms other than protein synthesis inhibition (Froscio et al. 2003; Humpage et al. 2005; Norris et al. 2002; Norris et al. 2001).

In the Reisner et al. (2004) report, microscopic examination of blood samples showed the presence of red blood cells with spiked surfaces rather than their normal biconcave-disc shape. The authors attributed the acanthocyte formation to an increase in the cholesterol to phospholipid ratio of the red blood cell membrane. Phospholipids constitute the matrix material of cell membranes. The authors hypothesized that this change was the consequence of decreased activity of plasma lecithin cholesterol acyl transferase, an enzyme associated with high-density lipoproteins and the esterification of plasma cholesterol. Effects on the cholesterol content of the red blood cell membrane can occur with inhibition of the enzyme increasing membrane fluidity and mean corpuscular volume. Removal of the abnormal blood cells by the spleen increases both spleen weight and serum bilirubin as well as stimulates hematopoiesis. Additional research is needed to examine the lecithin cholesterol acyl transferase enzyme inhibition hypothesis in order to confirm whether it accounts for the effects on the red blood cell as a result of cylindrospermopsin exposure.

Kidney necrosis and a decreased renal failure index at the high cylindrospermopsin doses provide support for the effects on the kidney. Numerous signs of renal damage including proteinuria, glycosuria, and hematuria were observed after a hepatoenteritis-like outbreak in Palm Island, Australia in 1979 (Byth 1980). The outbreak was attributed to consumption of drinking water with a bloom of *C. raciborskii*, a cyanobacteria that can produce cylindrospermopsin. These effects are associated with impaired kidney function (Byth 1980); however, no mode of action information for kidney effects was observed in the available animal studies of cylindrospermopsin. Since all the studies were conducted in mice, a species that excretes low molecular weight proteins in urine, there is a need to conduct a study of cylindrospermopsin in a laboratory species that does not excrete protein in the urine in order to determine whether there are comparable effects on kidney weight, protein excretion, and renal cellular damage.

5.1.2 Cancer

5.1.2.1 Weight of Evidence Classification

While there is evidence of an association between liver and colorectal cancers in humans and microcystins exposure and some evidence that microcystin-LR is a tumor promoter in mechanistic studies, there is inadequate information to assess carcinogenic potential of microcystins in humans (U.S. EPA 2005). The human studies are limited by lack of exposure information and the uncertainty regarding whether or not these studies adequately controlled for confounding factors such as hepatitis B infection. No chronic cancer bioassays for microcystins in animals are available. U.S. EPA (2005) states that the descriptor of "*inadequate information to assess carcinogenic potential*" is appropriate when available data are judged inadequate for applying one of the other descriptors or for situations where there is little or no pertinent information or conflicting information. The guidelines also state that (p. 2-52) "Descriptors can be selected for an agent that has not been tested in a cancer bioassay if sufficient other information, e.g., toxicokinetic and mode of action information, is available to make a strong, convincing, and logical case through scientific inference." In the case of microcystins, the data suggest that microcystin-LR may be a tumor promoter but not an initiator. Without stronger epidemiological data and a chronic bioassay of purified microcystin-LR, the data do not support classifying microcystin-LR as a carcinogen. The International Agency for Research on Cancer

(IARC) classified microcystin-LR as a Group 2B (possibly carcinogenic to humans) based on the conclusion that there was strong evidence supporting a plausible tumor promoter mechanism for these liver toxins (IARC 2010).

No chronic cancer bioassays of cylindrospermopsin were located in the literature. Limited data from an *in vivo* study showed no indication that the cyanobacterial extract containing cylindrospermopsin-initiated tumors in mice (Falconer & Humpage 2001).

5.2 Dose-Response Assessment

The RfD value for microcystin for this recreational AWQC is from EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* (U.S. EPA 2015d), where additional details are available. EPA identified a study by Heinze (1999) as the critical study in which male hybrid rats were administered microcystin-LR in drinking water at doses of 0 (n = 10), 50 (n = 10) or 150 (n = 10) µg/kg body weight for 28 days (Heinze 1999). The RfD of 0.05 µg/kg/d derived for microcystins was based on observed liver effects that included increased liver weight, slight to moderate liver necrosis lesions (with or without hemorrhages at the low dose and increased severity at the high dose), and changes in serum enzymes indicative of liver damage.

The RfD value for cylindrospermopsin for this recreational AWQC is from EPA's *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA 2015c), where additional details are available. EPA identified a study by Humpage and Falconer (2002; 2003) as the critical study in which male Swiss albino mice were administered purified cylindrospermopsin in water via gavage at doses of 0, 30, 60, 120, or 240 µg/kg/d for 11 weeks. The RfD of 0.1 µg/kg/d derived for cylindrospermopsin was based on increases in relative kidney weights along with indicators of reduced renal function effects at higher doses and decreased urinary protein.

6.0 SWIMMING ADVISORY AND RECREATIONAL CRITERIA DERIVATION

This section summarizes the inputs and shows the calculation for the recreational criteria and swimming advisory for microcystins and cylindrospermopsin.

6.1 Microcystins Magnitude

The magnitude of the swimming advisory and recreational criteria for microcystin toxins is calculated as follows:

$$\text{Recreational value (µg/L)} = \text{RfD} \times \frac{\text{RSC} \times \text{BW}}{\text{Ingestion Rate}}$$

Where:

RfD (µg/kg/d)	=	0.05 µg/kg/d (U.S. EPA 2015d)
RSC	=	0.8 (U.S. EPA 2000a)
BW (kg)	=	mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
Ingestion rate (L/d)	=	recreational water incidental ingestion rate for children (0.33 L/d) at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997)

$$\text{Microcystins recreational value} = 0.05 \text{ µg/kg/d} \times \frac{0.8 \times 31.8 \text{ kg}}{0.33 \text{ L/d}} = 4 \text{ µg/L}$$

6.2 Cylindrospermopsin Magnitude

The magnitude of the recreational criteria and swimming advisory values for cylindrospermopsin is calculated as follows:

$$\text{Recreational value (µg/L)} = \text{RfD} \times \frac{\text{RSC} \times \text{BW}}{\text{Ingestion Rate}}$$

Where:

RfD (µg/kg/d)	=	0.1 (U.S. EPA 2015c)
RSC	=	0.8 (U.S. EPA 2000a)
BW (kg)	=	mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
Ingestion rate (L/d)	=	recreational water incidental ingestion rate for children (0.33 L/d), at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997)

$$\text{Cylindrospermopsin recreational value} = 0.1 \text{ µg/kg/d} \times \frac{0.8 \times 31.8 \text{ kg}}{0.33 \text{ L/d}} = 8 \text{ µg/L}$$

6.3 Recommended Swimming Advisory and Recreational Criteria for Microcystins and Cylindrospermopsin

Recreational criteria and the swimming advisory include a magnitude, duration, and frequency. Magnitude is the numeric expression of the maximum amount of the contaminant that may be present in a waterbody that supports the designated use, in this case protecting public health of recreators. Duration is the period of time over which the magnitude is calculated. Frequency of excursion describes the maximum number of times the pollutant may be present above the magnitude over the specified time period (duration). The magnitude, duration, and frequency in combination protect the designated use (in this case primary contact recreation). EPA requests public comment on all three of these recommendations.

The magnitude values are based on body weight and intake in children and are considered protective of adverse health effects for adults. To protect public health of swimmers at a beach, EPA recommends that the magnitude of the advisory value not be exceeded on any single day. For adoption as a recreational water quality criterion, EPA recommends using an excursion frequency of no greater than 10 percent of days per recreational season (up to one year), which is similar to recommendations for other recreational criteria (U.S. EPA 2012a). The 10 percent exceedance rate can help inform decisions on identifying impaired and threatened waters. The seasonal assessment period can take into consideration the temporal variability of HABs in the waterbody. For example, HABs can occur in some waterbodies earlier and later in the recreational season, while in other waterbodies HABs can occur and persist as long as conditions are conducive to their growth. HABs that produce toxins that last for extended periods or that reoccur across years when conditions are conducive to cyanobacterial growth can signify waterbodies with excessive nutrient loadings. EPA does not anticipate states using these cyanotoxin recommendations alone for developing load allocations for Total Maximum Daily Loads (TMDLs) or for Water Quality-Based Effluent Limits (WQBELs). For permitting purposes, cyanobacteria or their toxins are not typically present in permitted discharges. Permits are more likely to be written to address point source discharges of the causal pollutants, such as nutrients, on a waterbody-specific or watershed basis, where the permit writer has determined there is a reasonable potential for the causal pollutants in the discharge to cause or contribute to an exceedance of the cyanotoxin standards.

The recommended recreational criteria or swimming advisory values for the cyanotoxins microcystins and cylindrospermopsin are presented in Table 6-1.

Table 6-1. Recreational Criteria or Swimming Advisory Recommendations for Microcystins and Cylindrospermopsin

Application of Recommended Values	Microcystins			Cylindrospermopsin		
	Magnitude (µg/L)	Frequency	Duration	Magnitude (µg/L)	Frequency	Duration
Swimming Advisory	4	Not to be exceeded	One day	8	Not to be exceeded	One day
Recreational Water Quality Criteria		No more than 10 percent of days	Recreational season (up to one calendar year)		No more than 10 percent of days	Recreational season (up to one calendar year)

As an example:

- To protect swimmers, the concentration of total microcystins shall not exceed 4 micrograms per liter in a day.
- To protect the recreational use, the concentration of total microcystins shall not exceed 4 micrograms per liter more than 10 percent of days in a recreational season.

7.0 EFFECTS CHARACTERIZATION

7.1 Cyanobacterial Cells

Cyanobacterial cell densities can indicate the eutrophic status of a water body, especially when considering the frequency and severity of HAB occurrence (Yuan & Pollard 2015). Thus, cyanobacterial cell densities, especially the extent, severity, and frequency of blooms under environmental conditions conducive to cyanobacterial cell growth, are an indicator of the ecological health of a water body.

Cyanobacterial cells are associated with two distinct sets of health endpoints. First, cyanobacteria are associated with toxin-related endpoints. The second set of health effects associated with cyanobacterial cells are the inflammatory health endpoints including rashes, respiratory and gastrointestinal distress, and ear and eye irritation, which may be instigated by direct contact with the cells, bioactive compounds in the cyanobacteria not currently classified as toxins, or by contact with cyanobacteria-associated microbial commensals via dermal, oral and/or inhalation exposure routes (Eiler and Bertilsson 2004; Gademann and Portman 2008). Such effects have been observed in various health studies, including epidemiological and clinical studies and outbreak reports (Bernstein et al. 2011; Geh et al. 2015; Lévesque et al. 2014; Lin et al. 2015; Pilotto et al. 1997; Pilotto et al. 2004; Stewart et al. 2006a,b). Also, while not all cyanobacteria produce cyanotoxins, scientists have observed a relationship between cyanobacteria density and cyanotoxin concentration (Loftin et al. 2016b). Environmental conditions and ecosystem interactions also affect the production and release of cyanotoxins into ambient waters. Cyanobacterial cell densities can be an indicator of the potential of a bloom to produce cyanotoxins. While there is uncertainty and variability associated with the propensity of a bloom to produce cyanotoxins, cell densities can be used to estimate the potential for cyanotoxin concentrations to exceed the recommended values presented in section 6.

7.1.1 Cyanobacterial Cells Related to Inflammatory Health Effects

Various health studies, described in more detail in Appendix D, relate recreational exposure to cyanobacterial cells with specific health endpoints that can be described as acute inflammatory or allergenic reactions. It is possible that these endpoints could be related to other biological or biochemical mechanisms that are not yet understood. Studies have (1) examined the epidemiological relationships of recreational exposure to cyanobacteria in the water to recreator-reported symptomologies, (2) characterized the allergenic and dermal reactions to exposed animals and humans in clinical and *in vitro* studies, and (3) collated information on illness outbreaks associated with recreational exposure to HABs. However, the reported health endpoints and cyanobacterial density associated with the inflammatory response health outcomes are not consistent. Empirical study differences, such as study size, species, strains of cyanobacteria present, and measurement of possible co-exposures and the cyanobacterial densities associated with significant health effects, lead to uncertainties in determining what level of cyanobacteria result in a specific level of inflammatory responses in these studies. The lack of a described dose-response characterizing cell-related inflammatory health effects could suggest a “threshold” rather than a specific dose-response relationship (Cochrane et al. 2015; Stewart et al. 2006b). Allergy is an example of a threshold mechanism, meaning that there is a level of exposure (i.e., a threshold value) below which the development of sensitization and the elicitation of an allergic reaction will not occur. Defining accurate numerical values for threshold

exposure levels is difficult due to lack of validated methods and uncertainties about the mechanism of sensitization (Cochrane et al. 2015).

WHO recommends the use of cyanobacterial cell densities related to an increasing scale of the probability of adverse health effects in the *Guidelines for Safe Recreational Water Environments* (WHO 2003b). They also estimated microcystin concentrations that could be associated with these cell density levels. WHO used this approach to differentiate "*between the chiefly irritative symptoms caused by unknown cyanobacterial substances and the potentially more severe hazard of exposure to high concentrations of cyanotoxins, particularly microcystins.*" Therefore, WHO recommended a series of three guideline values associated with incremental severity and probability of health effects rather than a single guideline value. The WHO guideline values are:

- Low probabilities of adverse health effects (20,000 cells per mL) can be associated with irritative or allergenic effects from exposure to cyanobacterial cells (corresponding to 10 µg chlorophyll *a* per liter, under conditions of cyanobacterial dominance). The Pilotto et al. (1997) epidemiological study directly informed the derivation of this cut point. They also estimated that microcystin concentrations of 2–4 µg/L can be expected at this level.
- Moderate probability of the adverse health effects (i.e., 100,000 cells per mL) (equivalent to approximately 50 µg chlorophyll *a* per liter, if cyanobacteria dominate) is associated with an increased potential for irritative health outcomes and the potential for negative health impacts associated with exposure to higher cyanotoxin concentrations. The 100,000-cell cut point was informed by (1) modifying the value for the WHO drinking-water guideline for microcystin-LR for a recreational exposure scenario and (2) translating microcystin concentrations to cell densities based on the average microcystin content of *Microcystis* cells (equivalent to 20 µg microcystin/L). The WHO estimated that "*at a cell density of 100,000 cells per mL, there is the potential for some frequently occurring species (i.e., microcystis) to form scums,"* which can "*increase risks for bathers and others involved in body-contact water sports."*
- The high probability of adverse health effects category is associated with the elevated potential for exposure to cyanotoxins and the potential for severe health outcomes. "*The presence of cyanobacterial scum in swimming areas represents the highest risk of adverse health effects due to abundant evidence for potentially severe health outcomes associated with these scums"* (estimated at 50–100 µg microcystin/L).

Epidemiological studies, clinical studies, and recreational water outbreak reports were identified during searches of the publicly available and peer-reviewed scientific literature that characterized the human health effects associated with recreating in surface waters where cyanobacteria were present (see Appendix D). Although these epidemiological studies provide evidence for statistically significant associations between cyanobacterial cell densities and possible inflammatory or allergenic health endpoints (Lévesque et al. 2014; Lévesque et al. 2016; Lin et al. 2015; Pilotto et al. 1997; Stewart et al. 2006b), they do not provide consistent evidence of associations either at similar densities of cyanobacterial cells or with the associated health endpoints. The wide range in cyanobacterial cell densities associated with various health outcomes, either with specific health endpoints or with combined symptom categories, implies potential variability in the stressor occurrence. Differing cyanobacterial community composition

and proportions of the more allergenic, non-cyanotoxin-producing strains relative to the cyanotoxin-producing strains at each site is a factor. Additionally, potential variability in sensitivity in the study populations, differences among the specific sites studied (e.g., fresh versus marine beaches), and uncertainty with the potential confounding effects of other microbes that can co-occur with cyanobacteria were some uncertainties associated with these data. Additional uncertainties are described below.

The limited size of some studies could have affected the ability to detect significantly increased rates of illness in individual symptom categories (Pilotto et al. 1997; Stewart et al. 2006b). Small sample size can diminish the statistical power of the study and the ability to detect an association if one exists (Rothman et al. 2008). The incomplete characterization or consideration of frank or opportunistic pathogens that could co-occur with cyanobacteria in ambient waters also could complicate conclusions related to the etiologic agent of the reported symptoms (Lévesque et al. 2014; Lin et al. 2015; Pilotto et al. 1997; Stewart et al. 2006b).

Variability in the reported associations, including with the range of cyanobacterial cell densities reported and with specific symptom categories, affected the ability to identify a discrete cyanobacterial cell density value that would provide a consistent level of protection across different waters. Pilotto et al. (1997) reported a significant association with the occurrence of one or more symptoms, such as skin rashes, eye irritation, ear irritation, gastrointestinal distress, fever and respiratory symptoms, and exposure to > 5,000 cells/mL for > 1 hour. Lévesque et al. (2014) observed a significant increase in GI symptoms associated with recreational contact. The increase in GI symptoms was significant in the > 20,000-cells/mL and > 100,000-cells/mL categories, and the positive trend for increasing illness with increased cyanobacterial cell densities also was significant at $p = 0.001$. Pilotto et al. (1997), however, in discussing the significance of the trend of increasing symptom occurrence and with the 5,000 cells/mL cut point, specifically suggested that the 20,000 cell/mL threshold might be too high to be adequately protective of recreators (Pilotto et al. 1997). Lin et al. (2015) reported significant associations between respiratory symptoms and exposure to the 25th to 75th percentile range of cyanobacterial cells excluding picocyanobacteria (range 37–237 cells/mL) and between reported respiratory, rash, and earache symptoms and exposure to the highest quartile (range 237–1,461 cells/mL). The 1,461-cells/mL value was the highest cell density observed in that study (Lin et al. 2015).

Cyanobacterial cell densities reported in the literature are used by states to provide "safe to swim" decisions by state and local health departments (see Table 2-3 for a list of states with cyanobacterial cell density guidelines; see Appendix B for state guidelines and associated actions). Due to the uncertainties associated with delineating discrete cyanobacterial densities associated with a specified level of protection for recreators, EPA is not recommending CWA 304(a) criteria that include quantitative cyanobacterial cell densities predictive of the inflammatory or allergenic health outcomes because available data do not support a consistent quantitative dose-response relationship at this time. However, EPA recognizes that studies examining the potential health effects associated with exposure to cyanobacterial cells demonstrate that exposure to the cells—particularly via dermal and inhalation exposure—can be associated with numerous health endpoints potentially characterized as inflammatory responses.

7.1.2 Cyanobacterial Cells as Indicators for Potential Toxin Production

Available information suggests that cyanobacterial cell density could be used as an indicator of the potential for a cyanobacterial HAB to produce cyanotoxins at the concentrations discussed in section 6. Although EPA is not recommending criteria at this time that address inflammatory health effects based on cyanobacterial cell density, many states already use cell-based guidelines based on recommendations from the World Health Organization (WHO 2003a). States use the cell density information gleaned from their monitoring efforts to inform decision-making. Also, remote sensing techniques using satellite-based imagery to observe cyanobacterial blooms are of increasing interest to states (Schaeffer et al. 2012; 2013). This approach detects the level of chlorophyll *a* or phycocyanins in the water and converts that to a cell density estimate. Therefore, a cell density value corresponding to the cyanotoxin criteria value is needed to interpret the remote sensing data. Below, EPA has used a similar approach as WHO to calculate a cyanobacterial cell density with the potential to produce the cyanotoxin at the criteria concentration.

The WHO guidelines were developed for microcystin and cyanobacterial cell density at different probabilities of adverse health effects to support management of recreational waters. The WHO designated a low probability of adverse health effects category associated with the cyanobacterial cell-related inflammatory response health endpoints (see section 7.1.1). The probability of adverse health effects increased to moderate and high levels based on the risk associated with the potential of the cyanobacterial cells to produce microcystin. For example, at a level of 100,000 cyanobacterial cells per mL, WHO estimated that a concentration of 20 μg microcystin per L is possible if those cells were predominantly *Microcystis* sp. and each cell contained an average of 0.2 pg microcystin per cell (WHO 2003).

Using this approach, EPA calculates a cyanobacterial density associated with the recommended microcystins criteria/ swimming advisory concentration as follows:

$$\text{Cyanobacterial Cell Density (CCD)} = \frac{\text{Ambient cyanotoxin concentration (ACC)}}{\text{Cell toxin amount (CTA)}}$$

Where:

CCD = calculated cell density associated with a specific toxin concentration

ACC = specific toxin concentration target in ambient water (e.g., AWQC value)

CTA = amount of toxin produced in a cyanobacterial cell

For the microcystin produced by *Microcystis* sp.:

ACC = 4 μg/L; recommended recreational criteria value for microcystins

CTA = 0.2 pg/cell; reported mean concentration of microcystin in a cell of *Microcystis* species

Adding in the conversion factors to convert units, the equation is:

$$CCD = \frac{ACC\ (\mu g/L) \times 10^6\ pg/\mu g}{CTA\ (0.2\ pg/cell)} \times \frac{L}{1{,}000\ mL}$$

Adding in the values,

$$CCD = \frac{4\ \mu g/L \times 10^6\ pg/\mu g}{0.2\ pg/cell} \times \frac{1\ L}{1{,}000\ mL} = 20{,}000\ cells/mL$$

Thus, a *Microcystis* sp. cell density of 20,000 cells/mL has the potential to result in a microcystin concentration of 4 μg/L.

There is variability in the estimate of cyanotoxin concentrations associated with cell density. WHO acknowledged that various cyanobacterial species could contain more or less microcystin per cell. Species that contain more microcystin could result in much higher water-column concentrations of the cyanotoxin at a similar cyanobacterial cell density. Cyanobacterial community differences between locations could affect the level of cyanotoxin that is present. For example, WHO discussed that a bloom dominated by *Planktothrix* could result in 10 to 20 times higher water-column cyanotoxin concentrations given the same cell density (WHO 2003b). The same cell density applied at different locations could result in inconsistent levels of health protection for recreators at those locations.

EPA surveyed the published peer-reviewed scientific literature for information on the amount of microcystin and cylindrospermopsin produced by or contained in a cell from a variety of freshwater blooms reported around the world. Laboratory-based culture studies with numerous clones of *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii*, *Planktothrix agardhii*, and *Planktothrix rubescens* were also found. Many of these references also included either biomass-toxin conversions or graphic data which would support conversion factors from cyanobacterial cell density (expressed in a variety of units including cells L^{-1}, biovolume (μm^3) L^{-1}, chlorophyll *a* L^{-1}) to toxin concentrations for these species. Cyanotoxin concentration is generally related to cyanobacterial cell abundance, which is determined by nutrient availability (Welker 2008), so nutrient concentration is often correlated to cyanotoxin concentration. Information gleaned from this literature search also suggests that cyanotoxin amounts can vary with genetic factors (i.e., some isolates lack the genes involved in toxin production, physiological factors (e.g., growth rate, growth stage, photosynthetic rate, and allelopathic factors), trophic factors (e.g., grazing interactions), and environmental factors (e.g., temperature, salinity, carbon dioxide concentration, light intensity, macronutrient [i.e., nitrogen, phosphorus] and micronutrient [e.g., trace metal concentrations]). Most data available are for microcystins rather than for cylindrospermopsin. Please refer to Appendix F for additional information on cyanotoxin amounts per cell and conversion factors found in the literature survey.

States that currently have guidelines for HABs in recreational waters consider cell densities, cyanotoxin concentrations, or both. Decisions to issue recreational water warnings/advisories, or initiate monitoring for cyanotoxins based on the cyanobacterial cell density only once a bloom is observed (i.e., green, discolored water and/or scum formation/accumulation associated with high densities of cells) may overlook situations where extracellular toxins are present. Cells may accumulate in locations different from where the bloom originated (e.g., by wind and/or wave action, or transport downstream). A cell density of

20,000 cells/mL (corresponding to the recommended AWQC value) is lower than that typically associated with a bloom (WHO 2003b). Decision points contingent on visually confirmed blooms may miss or delay the identification of the hazardous condition associated with exposure to elevated cyanotoxins. States may wish to consider using visual identification of blooms preferentially for waterbodies with a previous history of HAB events and/or microcystin detections.

7.2 Enhanced Risk or Susceptibility

Children recreating are likely to spend more time in direct contact with waters and measured incidental ingestion data while swimming indicate that children between 6 and 11 years ingest on average more water than older children and adults (Evans et al. 2006). No measured incidental ingestion data are available for children younger than 6 years old. A study by Schets et al. (2011), described in more detail in section 7.3, provides incidental ingestion volumes for children ages 0 to 14 years, but this study relied on surveyed parents' estimates of the amount their children incidentally ingested. Although this study used a qualitative approach that is less certain than the studies that used analytical methods, Schets et al. (2011) identified an average incidental ingestion volume for children aged 0 to 14 years that was the same as the mean ingestion volume reported by Dufour et al. (2006) for children aged 6 to 18 years (37 mL). Children ages 5 to 11 years also tend to spend more time in the water compared to younger and older life stages (U.S. EPA 1997). The significant differences between life-stages in the volume of water ingested while recreating and duration of exposure can translate to increased risk of exposure to cyanotoxins for children compared to adults.

Based on the available studies in animals, individuals with liver and/or kidney disease may be more susceptible than the general population since the detoxification mechanisms in the liver and impaired excretory mechanisms in the kidney may be compromised. Data from an episode in a dialysis clinic in Caruaru, Brazil where microcystins (and possibly cylindrospermopsin) were not removed by treatment of dialysis water, identify dialysis patients as a population of potential concern in cases where the drinking water source was contaminated with cyanotoxins.

The data on red blood cell acanthocytes suggest that individuals that suffer from anemia (e.g., hemolytic or iron-deficiency) might be a potentially sensitive population. Several rare genetic defects such as abetalipoproteinemia (rare autosomal recessive disorder that interferes with the normal absorption of fat and fat-soluble vitamins from food) and hypobetalipoproteinemia are associated with abnormal red blood cell acanthocytes, which appears to result from a defect in expression of hepatic apoprotein B-100, a component of serum low density lipoprotein complexes (Kane & Havel 1989). Individuals with either condition might be sensitive to exposure to cylindrospermopsin.

Available animal data are not sufficient to determine if there is a definitive difference in the response of males versus females following oral exposure to microcystins. Fawell et al. (1999) observed a slight difference between male and female mice in body weight and serum proteins, but no sex-related differences in liver pathology. Available animal data are not sufficient to determine if there is a definitive difference in the response of males versus females following oral exposure to cylindrospermopsin.

7.3 Other Studies of Ingestion While Swimming

EPA used the recommended incidental ingestion while recreating values discussed in the *Exposure Factors Handbook* (2011), which cites Dufour et al. (2006) as the basis for its default recreational ingestion values. Dufour et al. (2006) measured the incidental ingestion of water while participants were swimming in a pool and found that children under the age of 18 ingested higher volumes of water while swimming than adults. The values presented in EPA's *Exposure Factors Handbook* (2011) adjusted the Dufour et al. (2006) data from a per event basis to an hourly ingestion rate.

In addition to Dufour et al. (2006), five other studies (Dorevitch et al. 2011; Evans et al. 2006; Schets et al. 2011; Schijven & de Roda Husman 2006; Suppes et al. 2014) evaluated recreation-associated incidental ingestion. See Table 7-1 for a summary overview of the available studies of incidental ingestion while recreating.

Evans et al. (2006) presented results from an observational study of incidental water ingestion during recreational swimming activities using the same methodology as the Dufour et al. (2006) pilot study. They cited the methods published in the Dufour et al. (2006) pilot study, which involved using cyanuric acid as an indicator of pool water ingestion to estimate the amount of water ingested by boys (n = 107) and girls (n = 80) ages 6–18 years who were directed to stay in the pool and actively swim for 45 to 60 minutes. Evans et al. (2006) reported that children ages 6–18 years incidentally ingested a mean volume of 47 mL per swimming event (boys: 48 mL/event; girls: 47 mL/event). Consistent with Dufour et al. (2006), Evans et al. (2006) found that children ingested higher volumes of water than both adults and the entire study population combined. Adults (both genders combined) incidentally ingested a mean volume of 24 mL. Adult men and adult women incidentally ingested 30 mL and 19 mL, respectively. The entire study population had a mean incidental ingestion volume of 32 mL. The Evans et al. (2006) reported study has not been peer reviewed.

Suppes et al. (2014) evaluated incidental water ingestion rates using cyanuric acid as an indicator of pool-water ingestion, and found that children on average ingested pool water at a higher rate than adult participants. Total time in water, quantified by viewing videos, was used to adjust pool-water ingestion volumes to obtain rates. After adjustments for false-positive measurements were applied, the mean rate at which adults ingested water was 0.0035 L/hr with range 0–0.051 L. The mean rate at which children ingested water was 0.026 L/hr with range 0.0009–0.106 L/hr.

Taking a different approach, a study in the Netherlands by Schets et al. (2011) used questionnaires to collect estimates of water swallowed while swimming/bathing in freshwater, marine water, and swimming pools and found children had higher ingestion volumes. Two rounds of surveys were conducted, one in 2007 and another in 2009. Of the 8,000 adults who completed the questionnaire, 1,924 additionally provided estimates for their eldest child (< 15 years of age). The participants estimated the amount of water they or their children swallowed while swimming. Schets et al. (2011) also conducted a series of experiments to measure the amount of water that corresponded to a mouthful of water and converted the data in the four response categories to volumes of water ingested. Depending on the water type, adult men swallowed, on average, 0.027–0.034 L per swimming event and women swallowed 0.018–0.023 L. Children swallowed more than adults on average, 0.031–0.051 L per swimming event (Schets et al. 2011). Although the incidental ingestion data reported by Schets et al. (2011) were

based primarily on participant-reported estimates, the mean values were similar to those reported in Dufour et al. (2006).

Schijven and de Roda Husman (2006) studied sport and occupational diver incidental ingestion. The types of water studied for occupational divers (n = 37 divers) were open sea and coastal marine water, and freshwater. For sport divers (n = 483 divers), the types of water considered were open sea and coastal marine water, fresh recreational water, canals and rivers, city canals, and swimming pools. The divers were asked to estimate how much water they swallowed in terms of: none, few drops, shot glass, coffee cup, or soda glass. The authors translated the description of volumes from the questionnaires into average volumes. Occupational divers reported incidentally ingesting more water per dive in marine water (mean: 0.0098 L/dive; maximum: 0.1 L/dive) compared to freshwater (mean: 0.0057 L/dive; maximum: 0.025 L/dive). Sports divers wearing an ordinary diving mask reported incidentally ingesting the most water per dive in swimming pools (mean: 0.02 L/dive; maximum: 0.19 L/dive), followed by recreational freshwater (mean: 0.013 L/dive; maximum: 0.19 L/dive) and coastal marine water (mean: 0.0099 L/dive; maximum: 0.19 L/dive). Sports divers wearing a full face mask reported incidentally ingesting less water than sports divers wearing an ordinary diving mask. The age of the divers was not included in the study report. Duration of dives was also not reported.

Dorevitch et al. (2011) evaluated incidental ingestion associated with multiple types of water contact activities in both surface water and in pools. Volume of ingestion was self-reported via interviews (3,367 participants), and the authors used a subset of the pool exposures to assess cyanuric acid in urine to determine the accuracy of the self-reported ingestion volumes. There was strong agreement between self-reported results and cyanuric acid measurement (none = 0.0014±0.008 L; drop to teaspoon = 0.0094±0.011 L; mouthful = 0.026±0.037 L). In surface water, participants ages 6 and above incidentally ingested the most water while canoeing and capsizing compared to any other activity assessed (median = 0.0036 L; mean = 0.006 L; Upper 95 percent CI = 0.0199 L). In swimming pool water, participants ages 6 and above incidentally ingested the most water while swimming compared to any other activity assessed (median = 0.006 L; mean = 0.01 L; Upper 95 percent CI = 0.0348 L). Swimmers in a pool were more than 50 times as likely to report swallowing a teaspoon of water compared to people who canoed or kayaked in surface waters. Duration of activities was not reported, so the ingestion volumes are on a per event basis.

Additional estimates of incidental water ingestion rates while swimming in pools have been identified by EPA's Office of Pesticide Programs (OPP). OPP calculates people's exposures to pool chemicals while they swim using its SWIMODEL (U.S. EPA 2003). SWIMODEL uses incidental ingestion values for children that are twice the values used for adults. Incidental ingestion rates among adults while swimming competitively and noncompetitively are 0.0125 L/hr and 0.025 L/hr, respectively. The model assumes an incidental ingestion rate of 0.050 L/hr for children ages 7–10 and 11–14 years while swimming noncompetitively. The 0.050-L/hr value is the value used in EPA OPP's Standard Operating Procedures (2000b) and is based on recommendations from EPA's *Risk Assessment Guidance for Superfund*, Part A (ACC 2002; U.S. EPA 1989; U.S. EPA 1997; U.S. EPA 2000b; U.S. EPA 2003). SWIMODEL assumes that noncompetitive swimmers incidentally ingest water at twice the rate as competitive swimmers, which is based on recommendations from ACC (2002), which is unpublished.

Table 7-1. Studies of Incidental Ingestion Volumes or Rates While Recreating

Reference	Number of Participants, Water Type	Recreational Activity	Measurement Methodology	Measure-ment Parameter	Parameter Provided	Age Group(s)	Value	Mean Duration of Event	Mean Rate of Ingestion (mL/hr)
Dufour et al. (2006)	n = 53 Swimming pool	Swimming	Cyanuric acid was measured in pool water and urine samples	Ingestion volume per event	Mean	Children (6–<18 years old)[a]	37 mL	≥ 45 min	49
						Adults	16 mL		21
						All ages	32 mL		43
Evans et al. (2006)	n = >500 Swimming pool	Swimming	Cyanuric acid was measured in pool water and urine samples, and ingestion rate was calculated based on duration of swimming event	Ingestion volume per event	Mean (upper 95 percent CI)	Children (6–18 years old)[a]	47 mL (142 mL)	≥ 45 min	63
					Mean (95 percent CI)	Adults	24 mL (2–84 mL)		32
				Ingestion rate	Mean	6–15 years	42 mL/hr		42
						16+ years	28 mL/hr		28
						Children and adults	33 mL/hr		33
Dorevitch et al. (2011)	n = 3,367 Surface water	Canoeing and capsizing	Estimates of amount of water swallowed were self-reported	Ingestion volume per event	Median; Mean (upper 95 percent CI)	6+years[b]	3.6 mL; 6 mL (19.9 mL)	No duration constraints	-
		Kayaking and capsizing					2.9 mL; 5 mL (16.5 mL)		-
	Swimming pool	Swimming	Estimates of amount of water swallowed were self-reported; cyanuric acid was measured in urine in a subset of participants				6.0 mL; 10 mL (34.8 mL)	60 min	10
		Kayaking and capsizing					4.8 mL; 7.9 mL (26.8 mL)		7.9
		Canoeing and capsizing					3.9 mL; 6.6 mL (22.4 mL)		6.6
Schets et al. (2011)	n = 8,000 adults, 1,924 children Freshwater	Swimming	Descriptive estimates of the amount of water swallowed were self-reported by participants or parents of participants, and estimates were converted to volumes	Ingestion volume per event	Mean (95 percent CI)	0–14 years[a]	37 mL (0.14–170 mL)	79 min	28
						Adults, males	27 mL (0.016–140 mL)	54 min	30
						Adults, females	18 mL (0.022–86 mL)		20
	Marine water					0–14 years[a]	31 mL (0.08–140 mL)	65 min	29
						Adults, males	27 mL (0.016–140 mL)	45 min	36

Reference	Number of Participants, Water Type	Recreational Activity	Measurement Methodology	Measure-ment Parameter	Parameter Provided	Age Group(s)	Value	Mean Duration of Event	Mean Rate of Ingestion (mL/hr)
	Swimming pool					Adults, females	18 mL (0.022–90 mL)	41 min	26
						0–14 years[a]	51 mL (0.62–200 mL)	81 min	38
						Adults, males	34 mL (0.022–170 mL)	68 min	30
						Adults, females	23 mL (0.033–110 mL)	67 min	21
Suppes et al. (2014)	n = 38 Swimming pool	Swimming	Cyanuric acid was measured and total time in water was quantified using videos to adjust ingestion volumes to rates; authors adjusted Ingestion volumes to correct for potential false positive measurements from cyanuric acid carry-over between sample injections	Ingestion rate, adjusted	Mean (Standard deviation); Range	Children (5–17 years old)[a]	26 mL/hr (29 mL/hr); 0.9–106 mL/hr	≥ 45 min	26
						Adults	3.5 mL/hr (11.7 mL/hr); 0–51 mL/hr		3.5
						Children and adults	14 mL/hr (24 mL/hr); 0–106 mL/hr		14
				Ingestion rate, unadjusted	Mean; Maximum	Children (5–17 years old)[a]	59 mL/hr; 225 mL/hr		59
					Mean	Adults	9 mL/hr		9
						Children and adults	32 mL/hr		32
Schijven and de Roda Husman (2006)	n = 37 Freshwater	Diving, occupational	Descriptive estimates of the amount of water swallowed were self-reported, and estimates were converted to volumes	Ingestion volume per event	Mean; Maximum	Adults	5.7 mL; 25 mL	60–95 min	3.6–5.7
	Marine water						9.8 mL; 100 mL		6.2–9.8
	Coastal marine water[4]						12 mL; 100 mL		7.6–12
	n = 483 Swimming pool	Diving, recreational with ordinary diving mask					20 mL; 190 mL	42–52 min	23–29
	Recreational freshwater						13 mL; 190 mL		15–19
	Coastal marine water						9.9 mL; 190 mL		11–14
	Swimming pool						13 mL; 190 mL		15–19

Reference	Number of Participants, Water Type	Recreational Activity	Measurement Methodology	Measure-ment Parameter	Parameter Provided	Age Group(s)	Value	Mean Duration of Event	Mean Rate of Ingestion (mL/hr)
	Coastal and open marine water	Diving, recreational with full face mask					1.3 mL; 15 mL		1.5–1.9

[a] Data cannot be separated by different age groups among children.
[b] Results were not reported in children and adult categories.

Although these studies used different methodologies and have limitations with respect to reporting information for different age group categories, their results corroborate the Dufour et al. (2006) data. Similar to Dufour et al. (2006), the studies that included children confirmed that children ingested more than adults. The freshwater ingestion results reported by Schets et al. (2011) included parent estimates of children's ingestion of water while swimming in freshwater that are most similar to the Dufour et al. (2006) findings. Schets et al. (2011) found a mean ingestion volume for children aged 0 to 14 years of 37 mL, which is the same as the mean ingestion volume reported by Dufour et al. (2006) for children. The adult self-reported ingestion volumes in Schets et al. (2011) were also similar to the Dufour et al. (2006) adult value. Schets et al. (2011) reported adult values ranging from 18 and 27 mL for females and males, respectively, while Dufour et al.'s adult ingestion volume was 16 mL. Schijven and de Roda Husman (2006) found adult divers mean ingestion volumes while diving recreationally in a swimming pool or in freshwater ranged between 13 and 20 mL, varying depending on mask type used. Dorevitch et al. (2011) also evaluated self or parent estimates of ingestion volumes while swimming and found a mean ingestion volume for all ages of 10 mL. Suppes et al. (2014) used a similar measurement method as Dufour et al. (2006), i.e., measuring cyanuric acid as an indicator of pool water ingestion, to estimate the amount of water ingested by 16 children ages 5 to 17 years. After adjustment for false positives, the mean rate at which child participants ingested water was 26 mL/hr, just about half of the Dufour et al. (2006) normalized ingestion rate of 50 mL/hr.

7.4 Distribution of Potential Recreational Health Protective Values by Age

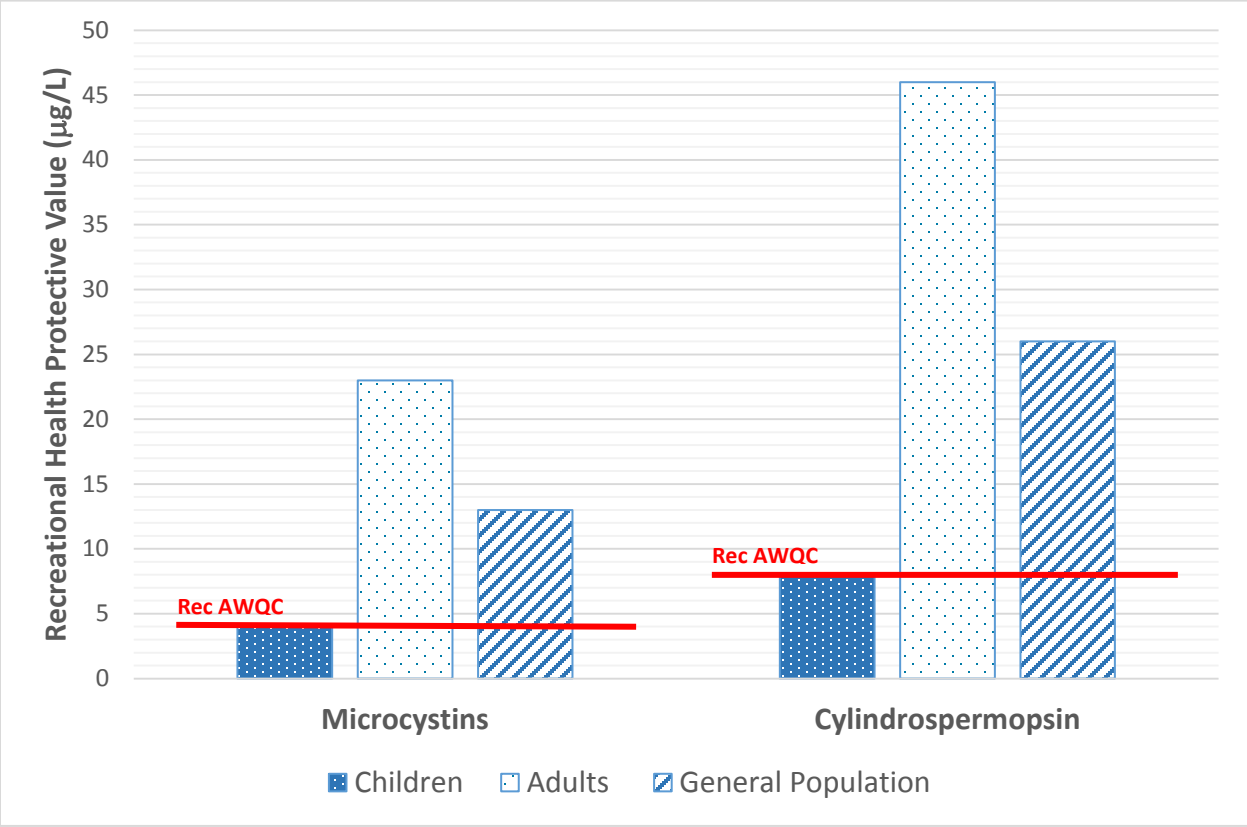
To evaluate the parameters used to calculate the cyanotoxin recreational AWQC, EPA compiled and evaluated available information for various lifestages. This section discusses potential health protective values for children and adults based on alternative data sets (section 7.4.1) and considers younger children's exposure parameters (section 7.4.2).

7.4.1 Evaluation of Criteria Related Lifestages

Using the ingestion rates for each age-group from EPA's *Exposure Factors Handbook* (U.S. EPA 2011), EPA estimated recreational health protective values for microcystins and cylindrospermopsin (plotted on Figure 7-1) to demonstrate the variability due to body weight, recreational water incidental ingestion, and exposure duration by lifestage.

EPA derived the recreational AWQC based on children's recreational exposures because this life stage has higher recreational exposures relative to adult recreators and the general population as a whole (i.e., all ages). As Figure 7-1 demonstrates, the calculated values for children (4 µg/L for microcystins and 8 µg/L for cylindrospermopsin) are protective of adults and the general population. EPA calculated for comparison recreational health protective values for adults using (1) 80 kg as the body weight (U.S. EPA 2011), (2) the maximum observed incidental ingestion value for adults (0.07 L/h) which EPA's *Exposure Factors Handbook* (2011) recommended due to the limited size of the data set, and (3) the mean recreational exposure duration for the 18- to 64-year age group (2.0 hr/d) (U.S. EPA 1997). The estimated recreational health protective values for adults are 23 µg/L for microcystins and 46 µg/L for cylindrospermopsin, Therefore, the recreational criteria and swimming advisories EPA calculated to be protective of children are protective of adults.

Figure 7-1. Comparison of Recreational Health Protective Values for Microcystins and Cylindrospermopsin for Children, Adults, and General Population



The parameters used to calculate health protective values for children include incidental ingestion values for children less than 18 years, mean body weight for children ages 6 to 11 years, and recreational exposure duration for children ages 5 to 11 years. The Dufour et al. (2006) incidental ingestion data are limited to children older than 6 years but less than 18 years. Schets et al. (2011) surveyed individuals 15 years and older to estimate their incidental ingestion of freshwater while recreating and asked those who had children to estimate incidental ingestion of their oldest child aged 0 to < 15 years. The ingestion volumes were initially binned into exposure classes and then translated into volumes using the results of a second study that quantified the distribution of volumes associated with ingested mouthfuls of water (Schets et al. 2011). The Schets et al. (2011) results for ages 0 to < 15 years were similar to estimates for 6 to < 18 years in Dufour et al. (2006). In both studies, children ingested more than adults on average and in the range of volumes (see Table 7-1). Based on the qualitative nature of the data available for the youngest children and given that the mean values were similar, EPA concludes that the values reported in the *Exposure Factors Handbook* are protective of children of all ages, including those younger than 6 years.

Evans et al. (2006) reported results of a full-scale study using the same methodology reported in Dufour et al. (2006); results of this study were presented by Evans et al. (2006) at an EPA recreational waters conference in 2006. The full-scale study included a study population sufficient to break out age categories that included younger children (6 to 10 years old), older children (11 to 17 years old), and adults. The number of study participants in the Dufour et al.

(2006) pilot study was 53, while their full-scale study evaluated more than 500 participants (Evans et al. 2006). Similar to the results reported in the pilot study, children (< 18 years old) ingested significantly more than adults. Additionally, Evans et al. (2006) reported that younger children (6 to 10 years old) ingest significantly more than older children (11 to 17 years old), or adults. Data quality standards require EPA to use independently peer reviewed and published data within our recommendations. Until this data set is published, EPA cannot include it in its analysis.

Table 7-2 presents a comparison of the daily ingestion rates (i.e., hourly ingestion rate times the exposure duration in hours) for the Dufour et al. (2006) study compared to Evans et al. (2006). While rates for "children" (< 18 years old) are similar between the studies, the ingestion rates using information from the newer study and duration rates from the EPA's *Exposure Factors Handbook* (2011) indicate a significantly higher exposure for younger children compared to older children or adults. A Kruskal-Wallis statistical test indicated that ingestion rates differed significantly between groups (p-value < 0.001). The pairwise Wilcoxon test with Bonferroni correction also indicated that ingestion rates in younger children (aged 6 to 10) were significantly different from ingestion rates in older children (p-value < 0.001). However, there is no difference between ingestion rates between older children (aged 11 to 17) and adults (p-value > 0.05).

Table 7-2. Comparison of Daily Ingestion Rates While Recreating between Dufour et al. (2006) and Evans et al. (2006)[a]

Age Group	Parameter Type[b]	Dufour et al. (2006) Based Daily Ingestion Rate (L/d)		Evans et al. (2006) Based Daily Ingestion Rate (L/d)		
Children	Mean	6 to < 18 years	0.13	0.14	6 to 10 years	0.22
					11 to 17 years	0.09
	Upper percentile		0.33	0.34	6 to 10 years	0.50
					11 to 17 years	0.23
Adults	Mean	18+ years	0.04	0.06		
	Upper percentile		0.14	0.12		

[a] The results reported in Evans et al. (2006) are not yet published. It is EPA's policy to use peer-reviewed study results to inform its regulatory efforts. The Evans et al. (2006) results are included within this effects characterization to provide context to the parameter values EPA used in the criteria derivation and because these results were presented publicly at the National Recreational Water Conference in 2006.

[b] The calculations of daily ingestion rate all used the mean exposure duration; the parameter type refers to the hourly ingestion rate.

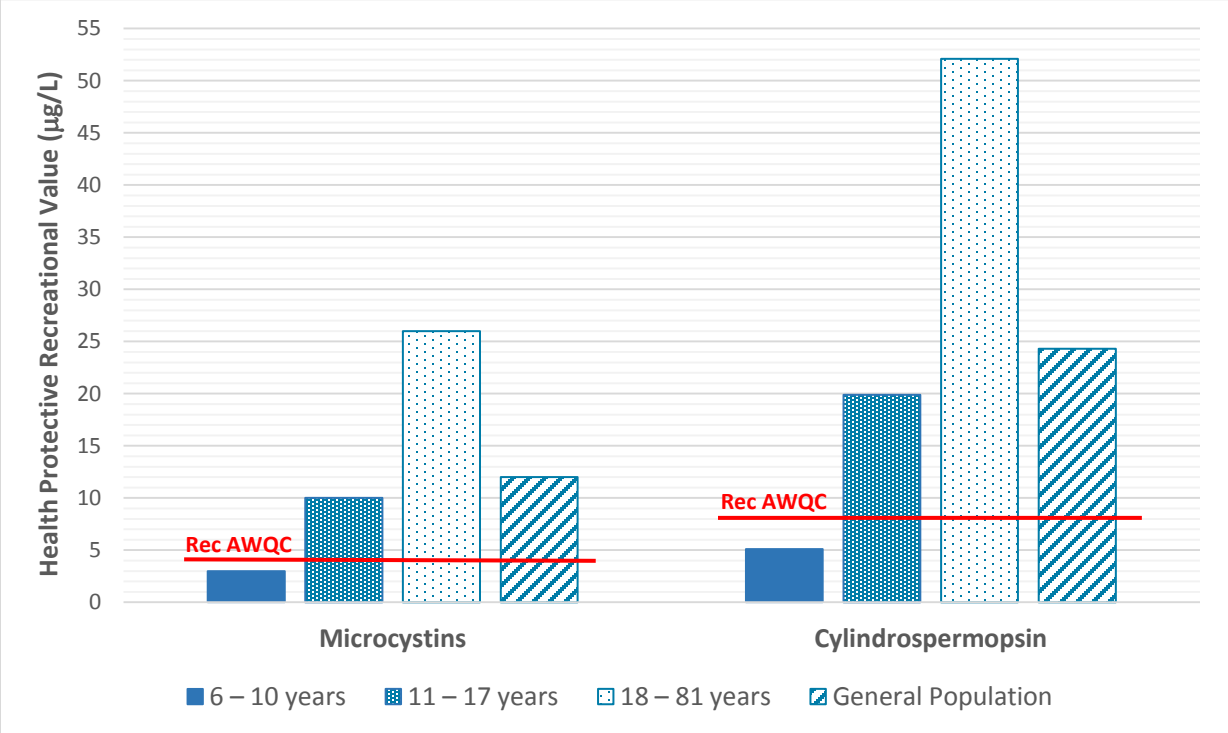
Table 7-3 presents alternative recreational values derived based on the more specific children's age groups available from Evans et al. (2006). Figure 7-2 provides a chart of this information for comparison purposes.

Table 7-3. Alternative Recreational Criteria Values for Microcystins and Cylindrospermopsin Calculated based on Alternative Ingestion Data from Evans et al. (2006)

Age Group	Body Weight (kg)	Ingestion Rate (L/d)[a]	Alternative Health Protective Recreational Cyanotoxin Value (µg/L)	
			Microcystins	Cylindrospermopsin
Children 6 to 10 years	31.8	0.50	3	5
Children 11 to 17 years	56.8	0.23	10	20
Adults 18 to 64	80	0.12	26	52
General Population	60	0.20	12	24

[a] Ingestion rate is the product of incidental ingestion volume normalized to one hour (L/hr) and the recreational duration (hr/d). Scenario uses mean body weight and ingestion rate based on upper percentile or maximum value for ingestion rate from EPA's *Exposure Factors Handbook* (U.S. EPA 2011) and mean value for recreational exposure duration in the older version of EPA's *Exposure Factors Handbook* (U.S. EPA 1997).

Figure 7-2. Comparison of Alternative Health Protective Recreational Values and Recreational AWQC for Microcystins and Cylindrospermopsin Calculated based on Evans et al. (2006)[a]



[a] The results reported in Evans et al. (2006) are not yet published. It is EPA's policy to use peer-reviewed study results to inform its regulatory efforts. The Evans et al. (2006) results are included within this effects characterization to provide context to the parameter values EPA used in the criteria derivation and because these results were presented publicly at the National Recreational Water Conference in 2006.

7.4.2 Evaluation of Younger Children's Exposure Factors

In the calculation of the cyanotoxin values reported in section 6, EPA utilized exposure parameters reported in the *Exposure Factors Handbook* (U.S. EPA 1997; U.S. EPA 2011). Information on children's mean body weights were available for children's age groups including 0 to 1 year, as well as 1 to < 2 year, 2 to < 3 years, etc. Using the body weight data provided in U.S. EPA (2011), weighted mean for the age groups 0 to < 6 years and 1 to < 6 years were calculated.

The available values from the *Exposure Factors Handbook* (1997, 2011) for incidental ingestion volume and exposure duration, however, were limited to specific age ranges. For incidental ingestion, the data reported were limited to children 6 years old and older. U.S. EPA (2011) recommends using the 97th percentile ingestion volume for children < 18 years based on the Dufour et al. (2006) measured incidental ingestion volume normalized to 1 hour. The Dufour et al. (2006) study did not include children younger than 6 years. The 97th percentile is recommended because the study had a small number of participants. U.S. EPA (1997) provided a recreational exposure duration for children ages 1 to 4 years (1.4 hr/d). This duration is shorter than the duration for children ages 5 to < 11 years (2.7 hr/d). Values for exposure duration were not available for children younger than 1 year.

To evaluate potential health-protective water quality values specifically for children younger than 6 years, EPA searched for additional exposure parameter information in the peer-reviewed and published scientific literature. Table 7-4 shows data availability and differences between the exposure parameters used in the microcystin and cylindrospermopsin recreational AWQC calculation (ages 6 to < 11 years) and estimates for younger lifestages. The younger lifestages include children aged 0 to 6 years, children aged 1 to 6 years, and children younger than 1 year.

EPA found one other study that characterized incidental ingestion for children. Schets et al. (2011) reported incidental ingestion volumes for children ages 0 to < 15 years. However, the study did not further divide this cohort into younger children and older children. These data for children represent parental estimates of volumes of freshwater incidentally ingested by their children. The ingestion volumes were initially binned into exposure classes and then translated into volumes using the results of a second study that quantified the distribution of volumes associated with ingested mouthfuls of water (Schets et al. 2011). Because of the initial binning and then the translation step to arrive at a distribution of ingestion volume for each exposure class, there is some uncertainty associated with the estimates. However, these estimates represent a different methodological approach compared to the approach used by Dufour et al. (2006). To facilitate comparing the results between the studies, EPA calculated an hourly incidental ingestion volume based on the Schets et al. (2011) data using the mean freshwater recreational durations reported in the same study. Because this study was not limited in size, as was the case with Dufour et al. (2006), EPA calculated both the 90th percentile and 97th percentile hourly ingestion volume. The 97th percentile was calculated to facilitate a more direct comparison with the results from Dufour et al. (2006) and the 90th percentile was calculated to provide a value that would typically be used to calculate health-protective values for a pollutant (U.S. EPA 2000a).

Table 7-4. Comparison of Younger Children's Exposure Factors and Incidental Ingestion Data Sets

Exposure Parameter	Used in Recreational AWQC Calculation	Dufour et al. (2006) Incidental Ingestion Data			Schets et al. (2011) Incidental Ingestion Data		
		Children 1 to < 6 years	Children 0 to < 6 years	Children < 1 year	Children 1 to < 6 years	Children 0 to < 6 years	Children < 1 year
Body weight (U.S. EPA 2011)	31.8 kg = mean body weight of children 6 to < 11 years	15.6 kg = weighted mean body weight of children 1 to < 6 years	13.4 kg = weighted mean body weight of children 0 to < 6 years	7.8 kg = weighted mean body weight of children 0 to < 1 year	15.6 kg = weighted mean body weight of children 1 to < 6 years	13.4 kg = weighted mean body weight of children 0 to < 6 years	7.8 kg = weighted mean body weight of children 0 to < 1 year
Incidental ingestion volume normalized to incidental ingestion per hour	0.12 L/hr = upper 97th percentile calculated based on study that included children 6 to < 18 years[a]				0.07 L/hr (90th percentile ingestion volume) 0.12 L/hr (97th percentile ingestion volume) calculated based on parent surveys for children 0 to < 15 years[b]		
Recreational exposure duration (U.S. EPA 1997)	2.7 hr/d = mean recreational exposure duration for children ages 5 to 11 years	1.4 hr/d = mean recreational exposure duration for children ages 1 to 4 years[c]					
Ingestion rate	0.33 L/d	0.17 L/d			0.10 L/d (90th percentile ingestion volume) 0.17 L/d (97th percentile ingestion volume)		

[a] Hourly ingestion rate for children is from EPA *Exposure Factors Handbook* (2011) Table 3-5: Ingestion of Water and Other Select Liquids from Dufour et al. (2006). The Dufour et al. (2006) pilot study measured incidental ingestion of water of participants who spent time swimming or playing in a swimming pool for at least a 45-minute period (n = 53; 41 children ages 6 to < 18 years; 12 adults > 18 years). This study did not include children younger than 6 years. U.S. EPA (2011) reported an hourly ingestion rate, which EPA calculated by normalizing the Dufour et al. (2006) ingestion volume per 45 minutes to an ingestion volume per hour (hourly ingestion rate) and also recommended using the 97th percentile as the "upper percentile" for children.

[b] Hourly ingestion rate for children is based on Schets et al. (2011) survey of Dutch parents' estimates of recreational duration and incidental ingestion volume while recreating in surface water; 486 of the survey respondents reported their children recreated in fresh water. The incidental ingestion volume 90th and 97th percentiles for children, 0.10 and 0.16 L/event, respectively, were calculated based on the distribution parameter reported in the paper. These volumes per event were normalized to an hourly ingestion rate by dividing these values by the mean duration per recreational event reported for children by Schets et al. (2011), which was 79 minutes or 1.3 hours.

[c] Recreational exposure duration values reported in EPA's *Exposure Factors Handbook* (1997) are limited to children > 1 year old. Children aged 1 to 4 years are the youngest life stage for which duration data are reported in Table 15-119 of U.S. EPA (1997).

When comparing the values for the normalized mean hourly ingestion volume (Table 7-4), both studies estimated a similar incidental ingestion volume of 0.12 L/hr. This is notable because Dufour et al. (2006) characterized the 6 to < 18-year age group and Schets et al. characterized the 0 to < 15-year age group. The similar volumes between the cohorts could indicate that the children younger than 6 years old were not contributing substantially to the distribution of incidental ingestion volume. Likewise, the same could be said for the 15 to 18-year age group. The results in Table 7-2 provide evidence that 6 to 10-year-old children incidentally ingest significantly more than 11 to < 18-year-old children or adults. The 90th percentile for incidental ingestion by 0 to < 15-year-old children reported by Schets et al. (2011) is approximately 40 percent lower than the 97th percentile volume for 6 to < 18-year-old children reported by Dufour et al. (2006).

EPA relied on the incidental ingestion volume recommended in the *Exposure Factors Handbook* (2011), which discusses the use of the 97th percentile ingestion volume reported in Dufour et al. (2006), because that study directly quantifies the water incidentally ingested while recreating. EPA included the Schets study in this discussion because it provides valuable context for characterizing children's incidental ingestion while recreating.

For children younger than 1 year, specific information is only available for body weights. Combining this parameter with ingestion volumes and exposure duration times reported for older age groups creates an exposure profile that would not seem to be representative of this early life stage. This combination of factors is presented in Table 7-4 for comparison purposes only. The available information is a better fit for children 1 to < 6 years.

Children 1 to 4 years are exposed for less time compared to children 5 to 11 years old, 1.4 hr/d compared to 2.7 hr/d, respectively (U.S. EPA 1997). Calculating the mean incidental ingestion rate per day for children younger than 6 years old based on results from Dufour et al. (2006) (0.17 L/d) or Schets et al. (2011) (0.10 L/d) results in lower estimated mean incidental ingestion rates compared to children ages 6 to < 11 years (Table 7-4). However, these estimates have large uncertainties given the lack of measured incidental ingestion data specifically for children younger than 6 years. Information on exposure durations for children < 1-year-old is also lacking. Because ingestion rates are greatest for 5 to 11 year olds, EPA concluded that calculating the ingestion rate using a higher duration was protective of children younger than 6 years old as indicated in Table 7-4 (Dufour et al. 2006; Schets et al. 2011).

7.5 Other Recreational Exposures

This section compares primary and secondary contact exposures and discusses tribal considerations for cyanotoxin and cyanobacterial cell exposure.

7.5.1 Other Recreational Exposure Pathways

EPA selected primary contact activities and incidental ingestion of water as the primary exposure pathway for derivation of the recreational criteria and swimming advisories. Alternative exposure parameter data could be considered in this approach as described in section 7.4. In this section, EPA evaluated potential cyanotoxin exposures via inhalation of aerosols and dermal contact. Inhalation and dermal toxicity data were not available; however, there are limited available data to estimate inhalation and dermal exposure. EPA conducted analyses to estimate inhalation and dermal exposure and compared those estimates to incidental ingestion of the

cyanotoxins while recreating. Section 7.5.1.1 compares recreational ingestion and inhalation exposures to microcystins. Similarly, section 7.5.1.2 compares recreational ingestion and dermal exposure.

7.5.1.1 Inhalation of Cyanotoxins

Volatilization of microcystins and cylindrospermopsin from water to air is not expected due to their size and charges. Both cyanotoxins are rather large compared to volatile chemicals. Microcystins' acid groups are charged at the pH of normal surface waters. Cylindrospermopsin features both negative and positive changes and like other zwitterions, do not volatilize significantly into the air from water (Butler et al. 2012).

EPA did an analysis to determine if the criteria/swimming advisory values based on incidental ingestion are protective of recreational inhalation exposures. Although the recreational use is primary contact recreation, such as swimming, data are available for secondary contact activities such as jet skiing or boating and white-capped wave, bubble-bursting action, which can result in cyanotoxins becoming aerosols (microscopic liquid or solid particles suspended in air). Cheng et al. (2007) collected via personal samples and found that volunteers recreating on a lake with a 1 µg/L concentration in water were exposed to air concentrations of microcystin-LR of approximately 0.08 ng/m^3 in their breathing zone.

Using the information from Cheng et al. (2007) and inhalation exposure parameters provided in EPA's *Exposure Factors Handbook* (2011), EPA compared the microcystin inhaled dose (ng/d) to the ingested dose. The parameters and calculations for this analysis are presented in Table 7-5. Using conservative assumptions for inhalation rates (i.e., moderate intensity and 95th percentile) and inhalation exposure duration (i.e., 5 hr/d) and comparing with mean incidental ingestion rates, the estimated ingested dose is 151 times higher than the estimated inhaled dose for children and 43 times higher than the estimated inhaled dose for adults.

This analysis supports the conclusion that inhalation exposure is negligible compared to incidental ingestion while recreating. The inhalation *toxicity* is unknown for microcystin, but if it is equal to ingestion toxicity, the values based on oral ingestion should be protective of recreational inhalation exposures. EPA did not conduct a similar analysis for cylindrospermopsin because published measured air concentration data for this cyanotoxin were not available.

The California Environmental Protection Agency (CalEPA) came to a similar conclusion for water skiers (Butler et al. 2012). They cited Cheng et al. (2007) and noted that their results showed that a liter of water contains 700,000 to 800,000 times the amount of cyanotoxins as in a cubic meter of air. CalEPA calculated that this concentration is equivalent to 1.3 to 1.4 µL aerosolized microcystin/m^3. Compared to the ingestion assumptions used for swimmers in the calculation of their recreational guideline (i.e., 50 mL/hr), CalEPA calculated that a water-skier would have to inhale at least 35,000 m^3/hr while skiing to achieve a dose equal to the swimmer, which is 17,000 times the inhalation rate of a marathon runner. CalEPA concluded that a water skier would not inhale enough aerosol to receive a dose similar to what a swimmer gets from ingestion.

Table 7-5. Comparison of Recreational Exposure Ingested Dose to Inhaled Dose of Microcystin

Age Group	Inhalation Rate (m^3/min)[a]	Inhalation Rate per Hour (m^3/hr) [volume per min × 60 min/hr]	Duration of Inhalation Exposure per Day (hr/d)	Daily Inhalation Rate Adjusted for Duration of Exposure (m^3/d)	Concen-tration in Air (ng/m^3)[b]	Inhaled Dose (ng/day) [daily rate × conc. in air]	Inhaled Dose (μg/day)	Ingestion Rate (L/d)	Concen-tration in Water (μg/L)	Ingestion Dose (μg/day) [water conc. × daily ingestion rate]	Ratio of Ingested Dose to Inhalation Dose [ingested dose/inhaled dose]
Assumed 95th percentile short-term inhalation exposure, moderate intensity activity level inhalation rate (U.S. EPA 2011), a 24-hour inhalation exposure duration, and mean ingestion rate (U.S. EPA 2011), mean recreational exposure duration (U.S. EPA 1997), and water concentration of 1 μg/L (Cheng et al. 2007)											
Children	0.037	2.2	5.0	53	0.08	4.3	0.004	0.13	1	0.13	151
Adults	0.040	2.4	5.0	58	0.08	4.6	0.005	0.04	1	0.04	43

[a] EPA's *Exposure Factors Handbook* (2011) did not report recommended short term, moderate intensity activity level inhalation rate values for children or adults in aggregate; used highest inhalation rate listed for children and adult age groups for this conservative screen. For children, it was the age group 16 to < 21 years, and for adults, it was 51 to < 61 years.

[b] Cheng et al. (2007) measured 0.08 ng/m^3 in air near surface waters with a concentration of 1 μg/L microcystins. Assuming a linear relationship of water concentration to air concentration based on Cheng et al. (2007), the concentration in air at the recreational AWQC concentration for microcystins (i.e., 4 μg/L) is calculated by multiplying 4 μg/L by the ratio of (0.08 ng/m^3)/(1 μg/L), which equals 0.32 ng/m^3.

Another comparison considers spray exposures from jet-ski and boat spray. Sinclair et al. (2016) modeled a water-spray exposure scenario and observed much lower exposures than those resulting from swimming or limited-contact recreational activities reported in the previous study. Thus, EPA expects that the comparison above based on exposure from secondary contact recreation is protective of primary contact recreation. Sinclair et al. (2016) also measured urinary concentrations of cyanuric acid after 26 participants' exposure to spray in a simulated 10-minute car wash situation. Each subject wore a protective coverall with hood, vinyl gloves, waterproof footwear, and safety glasses to ensure that only their face and mouths were exposed. The estimated median and 90th percentile ingestion volumes were 0.18 and 1.89 mL, respectively. Converted to a duration of 1 hour, the amounts would be 1.08 mL and 11.3 mL, which are much lower than the incidental ingestion intakes per hour.

7.5.1.2 Dermal Absorption

EPA did not find any peer reviewed measured data for microcystin or cylindrospermopsin dermal absorption. EPA's *Dermal Exposure Assessment: A Summary of EPA Approaches* (U.S. EPA 2007) states that to get through the skin, a chemical must dissolve into the stratum corneum, which is a stabilized lipid barrier; therefore, lipid solubility is required initially (U.S. EPA 2007).

EPA used the dermal exposure equations in its *Risk Assessment Guidance for Superfund* (U.S. EPA 2004) to estimate the potential absorbed dose of microcystins and compare it to the incidentally ingested dose. Octanol-water partition coefficients required by these equations are available for four microcystins, including microcystin-LR. Ward and Codd (1999) estimated the log octanol-water partition coefficients of microcystin-LR, -LY, -LW and -LF using high performance liquid chromatography (HPLC) as 2.16, 2.92, 3.46, and 3.56, respectively. Cylindrospermopsin dermal absorption could not be predicted due to the lack of these lipophilicity parameters.

The equation to estimate skin permeability coefficient from U.S. EPA (2004) is

$$\text{Log } K_p = -2.80 + 0.66 \times \log K_{ow} - 0.0056 \times MW$$

Where:

K_p	=	Dermal permeability coefficient of compound in water (cm/hr)
K_{ow}	=	Octanol-water partition coefficient (dimensionless)
MW	=	molecular weight (g/mole)

The equation to estimate dermal absorbed dose for highly ionized organic chemicals from U.S. EPA (2004) is:

$$DA = K_p \times Cw \times t$$

Where:

DA	=	Absorbed dose per event (mg/cm^2-event)
K_p	=	Dermal permeability coefficient of compound in water (cm/hr)

Cw	=	Chemical concentration in water (mg/cm^3)
t	=	Event duration (hr/event)

The estimated microcystins absorbed dose based on these calculations and the exposure parameters used for microcystins are presented in Table 7-6. Although this analysis is based on very limited data, it supports the hypothesis that the dermal absorbed dose of microcystins is likely to be negligible compared to incidentally ingested doses during recreational activities.

CalEPA also concluded dermal absorption of microcystins and cylindrospermopsin while swimming is not expected to be significant due to the large size and charged nature of these molecules (Butler et al. 2012). CalEPA eliminated the dermal absorption pathway from its risk assessment of microcystins and cylindrospermopsin citing evidence that similarly large molecules such as antibiotics have not been able to be formulated in a way to penetrate the skin (Butler et al. 2012). A U.S. Army-contracted *in vitro* study by Kemppainen et al. (1990) measured microcystin dermal penetration in 48 hours through excised human abdominal skin and found 0.9 (±0.3) percent of the total dose in water penetrated through the skin; however, this study has not been peer reviewed.

7.5.2 Tribal Considerations

EPA considered alternative exposure scenarios tribal communities might have, given their cultural practices. Native American food foraging customs or cultural or religious ceremonies can put them into primary or secondary contact with cyanotoxins. Primary contact ceremonial use may include the use of a surface water body for religious or traditional purposes by members of a tribe, involving immersion and intentional or incidental ingestion of water (Eastman 2007).

It is uncertain whether these activities would lead to cyanotoxin exposures higher than the primary recreational contact assumptions for incidental ingestion and exposure duration used in this assessment.

7.6 Livestock and Pet Concerns

The world's first scientific report of adverse effects to animals from cyanobacteria was written by George Francis, who described in 1878 the rapid death of stock animals at Lake Alexandrina, a freshwater lake at the mouth of the Murray River in South Australia (Francis 1878). Since then, there have been numerous descriptions of mammal and bird mortalities associated with exposure to cyanobacteria (Backer et al. 2015; Hilborn & Beasley 2015). The literature throughout the 20th century includes reports from all inhabited continents (Stewart et al. 2008). However, the impacts of cyanotoxins on domestic and companion animals are likely under-recognized because many cases are misdiagnosed, few cases are biochemically confirmed, and even fewer are reported in the scientific literature or to animal health systems (Zaias et al. 2010).

Table 7-6. Comparison of Recreational Exposure Ingested Dose to Dermal Absorbed Dose of Microcystins

Microcystin	Log K_{ow} (Ward & Codd 1999)	Molecular Weight	Log Skin Perm-eability Coefficient (Log Kp)	Skin Perm-eability Coefficient (K_p) (cm/hr)	Chemical Conc. in Water (mg/cm^3) Assuming Rec AWQC Level	Event Duration[a] (hr/event) (mean for 5- to 11-year-olds)	Dermal Absorbed Dose per Event (mg/cm^2-event)	Total Body Surface Area (cm^2) (U.S. EPA 2011) 95th percentile Children 6 to < 11 Years	Dermal Absorbed Dose per Event (mg/event)	Dermal Absorbed Dose per Event (mg/event)	Ratio of Ingested Dose to Dermal Absorbed Dose
Microcystin-LR	2.16	995.17	-6.95	1.1E-07	4.00E-06	2.7	1.2E-12	1.48E+04	2E-08	2E-05	71,824
Microcystin-LY	2.92	1002.16	-6.48	3.3E-07	4.00E-06	2.7	3.6E-12	1.48E+04	5E-08	5E-05	24,764
Microcystin-LW	3.46	1025.2	-6.26	5.5E-07	4.00E-06	2.7	6.1E-12	1.48E+04	9E-08	9E-05	14,670
Microcystin-LF	3.56	986.16	-5.97	1.1E-06	4.00E-06	2.7	1.2E-11	1.48E+04	2E-07	2E-04	7,618

[a] Event duration is defined as 24-hour cumulative time spent at home in outdoor pool or spa as reported in EPA's *Exposure Factors Handbook* (U.S. EPA 1997).

Livestock and pets can potentially be exposed to higher concentrations of, or have increased exposure to, cyanotoxins than humans because they are known to consume cyanobacterial scum and mats and drink cyanobacteria-contaminated water (Backer et al. 2013). Dogs are additionally at risk, as they may lick cyanobacterial cells from their fur after swimming in a water body with an ongoing bloom. Mats and scums can represent thousand-fold to million-fold concentrations of cyanobacterial cell populations, and published microcystin concentrations have ranged up to 24 mg microcystin/L from scum material (Chorus & Bartram 1999). Common signs of HAB cyanotoxin poisonings in pets include repeated vomiting, diarrhea, loss of appetite, abdominal swelling, stumbling, seizures, convulsions, disorientation, inactivity, or skin rashes and hives (New York Sea Grant 2014; Trevino-Garrison et al. 2015). Although reports of livestock deaths are relatively rare, in extreme cases death can occur minutes after drinking from a contaminated water source. Acute symptoms of cyanotoxin poisoning can include loss of appetite, weakness, staggering, or inflammation of the muzzle, ear, or udder. Higher levels of cyanotoxins can lead to severe liver damage, the development of jaundice, and severe photosensitization. Often livestock or pets that recover from these ailments can then suffer from chronic failure to thrive (Australia Department of Economic Development Jobs Transport and Resources 2013; Robinson & Alex 1987).

7.6.1 States and Animal HAB Guidelines

A few states have guideline levels specific to the protection of animals from cyanotoxin poisoning (Appendix G). California has dog and cattle action levels for the cyanotoxins microcystin, anatoxin-a, and cylindrospermopsin (Butler et al. 2012). For both dogs and cattle, California estimated drinking water ingestion rates based on two publications by the National Research Council, Nutrient Requirements for Beef Cattle and Nutrient Requirements for Dogs and Cats. The animal specific RfD for each cyanotoxin was divided by the final water and cyanobacterial biomass intake exposure levels, providing a cyanotoxin concentration that would result in exposure at the RfD level or below. These calculations were performed for an acute (lethal) and a subchronic scenario. Oregon has dog-specific guideline values for the cyanotoxins anatoxin-a, cylindrospermopsin, microcystin, and saxitoxin based on the CalEPA method. However the dog-specific guideline value for saxitoxins was modified by applying an uncertainty factor for interspecies differences in sensitivity between humans (the species in the critical study) and dogs (Oregon Health Authority 2016). Grayson County in Texas gives information for domestic animals at current advisory levels for microcystin and cylindrospermopsin. Advisories levels of 20 ppb for microcystin and cylindrospermopsin are calculated as gallons of water that can be consumed for 10 and 80 pound dogs that will cause a lethal or near-lethal dose. This does not include additional dose amounts that could be ingested by a dog while self-grooming cyanobacteria scum off its fur (Lillis et al. 2012).

Other states mention animal poisoning in their guideline documents but do not give guideline values specific to livestock or companion animals. For example, Utah and Washington report that animal illness or death can be reason to issue or accelerate a HAB advisory warning (Hardy & Washington State Department of Health 2008; Utah Department of Environmental Quality and Department of Health 2015). However, Ohio issues the disclaimer that thresholds used are protective of human exposure and may or may not be protective of animals such as dogs or livestock (Kasich et al. 2015). Several other states including Connecticut, Idaho, Kansas, Massachusetts, Nebraska, Vermont, and Virginia provide informational pamphlets, warn about

harm to pets or other animals, or post about harm to animals in their beach warnings and advisory signage (Connecticut Department of Public Health: Connecticut Energy Environment 2013; IDEQ 2015; Kansas Department of Health and Environment 2016; Massachusetts Bureau of Environmental Health 2015; Nebraska Department of Environmental Quality and Nebraska Department of Health and Human Services: Division of Public Health 2016; Vermont Department of Health 2015; Virginia Department of Health 2012).

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APPENDIX A. INTERNATIONAL RECREATIONAL WATER GUIDELINES FOR CYANOTOXINS AND CYANOBACTERIA

Jurisdiction	Recreational Water Guideline Level	Recommended Action
Australia[a]	cyanobacteria (total): $\geq$ 10 mm^3/L (where known toxins are not present)	red level action mode; level 2 guideline: • Immediately notify health authorities for advice on health risk. • Make toxicity assessment or toxin measurement of water if this has not already been done. • Health authorities warn of risk to public health (i.e., the authorities make a health risk assessment considering toxin monitoring data, sample type and variability).
	cyanobacteria (total): $\geq$ 4 mm^3/L (where a known toxin producer is dominant in the total biovolume)	red level action mode; level 1 guideline: • Immediately notify health authorities for advice on health risk. • Make toxicity assessment or toxin measurement of water if this has not already been done. • Health authorities warn of risk to public health (i.e., the authorities make a health risk assessment considering toxin monitoring data, sample type and variability).
	cyanobacteria (total): $\geq$ 0.4 to < 10 mm^3/L (where known toxin producers are not present)	amber level alert mode: • Increase sampling frequency to twice weekly where toxigenic species are dominant within the alert level definition (i.e., total biovolume). • Monitor weekly or fortnightly where other types are dominant. • Make regular visual inspections of water surface for scums. • Decide on requirement for toxicity assessment or toxin monitoring.
	cyanobacteria (total): $\geq$ 0.4 to <4 mm^3/L (where a known toxin producer is dominant in the total biovolume)	amber level alert mode: • Increase sampling frequency to twice weekly where toxigenic species are dominant within the alert level definition (i.e., total biovolume). • Monitor weekly or fortnightly where other types are dominant. • Make regular visual inspections of water surface for scums. • Decide on requirement for toxicity assessment or toxin monitoring.
	cyanobacteria (total): $\geq$ 0.04 to <0.4 mm^3/L	green level surveillance mode: • Weekly sampling and cell counts at representative locations in the water body where known toxigenic species are present; or • Fortnightly for other types including regular visual inspection of water surface for scums.
	cyanobacterial scums consistently present	red level action mode; level 2 guideline: • Immediately notify health authorities for advice on health risk.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
		• Make toxicity assessment or toxin measurement of water if this has not already been done. • Health authorities warn of risk to public health (i.e., the authorities make a health risk assessment considering toxin monitoring data, sample type and variability).
	microcystins (total): ≥ 10 µg/L	red level action mode; level 1 guideline: • Immediately notify health authorities for advice on health risk. • Make toxicity assessment or toxin measurement of water if this has not already been done. • Health authorities warn of risk to public health (i.e., the authorities make a health risk assessment considering toxin monitoring data, sample type and variability).
	Microcystis aeruginosa (total): ≥ 50,000 cells/mL	red level action mode; level 1 guideline: • Immediately notify health authorities for advice on health risk. • Make toxicity assessment or toxin measurement of water if this has not already been done. • Health authorities warn of risk to public health (i.e., the authorities make a health risk assessment considering toxin monitoring data, sample type and variability).
	Microcystis aeruginosa (total): ≥ 5,000 to < 50,000 cells/mL	amber level alert mode: • Increase sampling frequency to twice weekly where toxigenic species are dominant within the alert level definition (i.e., total biovolume). • Monitor weekly or fortnightly where other types are dominant. • Make regular visual inspections of water surface for scums. • Decide on requirement for toxicity assessment or toxin monitoring
	Microcystis aeruginosa (total): ≥ 500 to < 5,000 cells/mL	green level surveillance mode: • Weekly sampling and cell counts at representative locations in the water body where known toxigenic species are present; or • Fortnightly for other types including regular visual inspection of water surface for scums.
Canada[d]	cyanobacteria (total): ≥ 100,000 cells/mL	issue swimming advisory
	detection of a cyanobacterial bloom	issue beach closure
	microcystins (total): ≥ 20 µg/L (expressed as microcystin-LR)	issue swimming advisory

Jurisdiction	Recreational Water Guideline Level	Recommended Action
Cuba[c]	any report of toxic effect in humans or animals	action (in red): as for "Alert", but with increased actions for public communication
	benthic mats: < 40 percent coverage of surfaces with any cyanobacteria; > 20 percent with toxicogenic cyanobacteria; > 50 percent with potentially toxicogenic cyanobacteria (particularly where they are visibly detaching and accumulating in scum)	alert: increased sampling (weekly and more sites); daily inspection; notification to public health unit and local managers; report to local government; warning of the public
	cyanobacteria: < 500 cells/mL	monthly visual inspection
	cyanobacteria: ≥ 1 of the species known as potentially toxic	alert: increased sampling (weekly and more sites); daily inspection; notification to public health unit and local managers; report to local government; warning of the public
	phytoplankton cells: ≥ 20,000 to < 100,000 cells/mL, > 50 percent of cells cyanobacteria	alert: increased sampling (weekly and more sites); daily inspection; notification to public health unit and local managers; report to local government; warning of the public
	phytoplankton: > 0 to < 1,500 cells/mL	monthly visual inspection and sampling at least four months per year
	scum consistently present; confirmed bloom persistence	action (in red): as for "Alert", but with increased actions for public communication
Czech Republic[c]	cells: > 100,000 cells/mL	2nd warning level: closure for public recreation
	cells: > 20,000 cells/mL	1st warning level (not otherwise specified)
Denmark[c]	chlorophyll *a*: > 50 μg/L, dominated by cyanobacteria	relevant authorities are informed and decide when and how the public should be informed; warnings include signs, media and contact to local user groups such as kindergardens, scouts, water sports clubs
	visible surface scum	relevant authorities are informed and decide when and how the public should be informed; warnings include signs, media and contact to local user groups such as kindergardens, scouts, water sports clubs
European Union[f]	cyanobacterial proliferation (occurrence)	when cyanobacterial proliferation occurs and a health risk has been identified or presumed, adequate management measures shall be taken immediately to prevent exposure, including information to the public
	cyanobacterial proliferation (potential for)	appropriate monitoring shall be carried out to enable timely identification of health risks.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
Finland[c]	algae (includes cyanobacteria): detected	level 1: Possibly microscopic examination and even toxin analysis if there is a specific cause such as very popular beach or reports of adverse health effects or animal deaths
	algae (includes cyanobacteria): high amount	level 2: Preferably microscopical examination; toxin analysis; warning of the public is compulsory
	algae (includes cyanobacteria): very high amount	level 3: Preferably microscopical examination; toxin analysis; warning of the public is compulsory
France[c]	bloom, scum, change in water color	microscopy examination. If cyanobacteria are absent: no further action. If present: counting and genus identification
	cyanobacteria: < 20,000 cells/mL (±20 percent)	active daily monitoring. Counting at least on a weekly basis. Normal recreational activity at the site
	cyanobacteria: > 100,000 cells/mL (±20 percent)	bathing and recreational activities are restricted. Public is informed.
	cyanobacteria: $\geq$ 20,000 to < 100,000 cells/mL (±20 percent)	active daily monitoring. Counting on a weekly basis. Recreationalactivities are still allowed; the public is informed by posters on site.
	microcystins (MC): 25 µg/L (±5 percent)	if MC < 25 µg/L bathing and recreational activities are restricted. If MC > 25 µg/L bathing is banned and recreational activities are restricted. In either case, public is informed.
	visible scum or foam in recreational or bathing area	all water activities in this area are prohibited. Restrictions do not necessarily apply to the whole recreational site. Other areas without scum may still be open.
Germany[c]	Secchi Disk reading > 1 m AND biovolume: < 1 mm^3/L	monitor further cyanobacterial development
	Secchi Disk reading > 1 m AND biovolume: $\geq$ 1 mm^3/L	publish warnings, discourage bathing, consider temporary closure
	Secchi Disk reading > 1 m AND chlorophyll *a* (with dominance by cyanobacteria): < 40 µg/L	monitor further cyanobacterial development
	Secchi Disk reading > 1 m AND chlorophyll *a* (with dominance by cyanobacteria): $\geq$ 40 µg/L	publish warnings, discourage bathing, consider temporary closure
	Secchi Disk reading > 1 m AND microcystins: < 10 µg/L	monitor further cyanobacterial development

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	Secchi Disk reading > 1 m AND microcystins: ≥ 10 µg/L	publish warnings, discourage bathing, consider temporary closure
	visible heavy scums and/or microcystins: > 100 µg/L	publish warnings, discourage bathing, temporary closure is recommended
Hungary[c]	cell count: ≥ 50,000 to < 100,000 cells/mL	no recommended actions listed, water body classification: Acceptable
	cell count: < 20,000 cells/mL	no recommended actions listed, water body classification: Excellent
	cell count: ≥ 20,000 to < 50,000 cells/mL	no recommended actions listed, water body classification: Good
	cell count: ≥ 100,000 cells/mL	no recommended actions listed, water body classification: Unacceptable
	chlorophyll *a* (with dominance by cyanobacteria): < 10 µg/L	no recommended actions listed, water body classification: Excellent
	chlorophyll *a* (with dominance by cyanobacteria): ≥ 10 to < 25 µg/L	no recommended actions listed, water body classification: Good
	chlorophyll *a* (with dominance by cyanobacteria): ≥ 25 to < 50 µg/L	no recommended actions listed, water body classification: Acceptable
	chlorophyll *a* (with dominance by cyanobacteria): ≥ 50 µg/L	no recommended actions listed, water body classification: Unacceptable
	microcystins: ≥ 4 to < 10 µg/L	no recommended actions listed, water body classification: Good
	microcystins: ≥ 10 to < 20 µg/L	no recommended actions listed, water body classification: Acceptable
	microcystins: < 4 µg/L	no recommended actions listed, water body classification: Excellent
	microcystins: ≥ 20 µg/L	no recommended actions listed, water body classification: Unacceptable
Italy[c]	cyanobacterial cell count (combined with identification of genus and, if possible, species): < 20,000 cells/mL	if possible, daily visual observation; weekly counting
	cyanobacterial cell count (combined with identification of genus and, if possible, species): > 100,000 cells/mL	bathing prohibited until quantification of microcystins; information to the public; at least weekly counting

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	cyanobacterial cell count (combined with identification of genus and, if possible, species): ≥ 20,000 to ≤ 100,000 cells/mL	daily visual observation; at least weekly counting; information to the public; quantification of microcystins
	microcystins: >25 µg/L	bathing prohibited
	visible scums	bathing prohibited until quantification of microcystins; warning notice; scum drift monitoring
Netherlands[c]	biovolume (cyanobacterial cell count): >0 to < 2.5 mm^3/L	surveillance level: continue fortnightly monitoring
	biovolume (cyanobacterial cell count): > 15 mm^3/L (if 80 percent dominance of microcystin-producers and microcystin < 20 µg/L, revert to Alert Level 1).	alert level 2: weekly monitoring and advice against bathing (by public authority): "You are advised not to bathe in this water;" prohibition by local authority is possible.
	biovolume (cyanobacterial cell count): ≥ 2.5 to ≤ 15 mm^3/L	alert level 1: weekly monitoring and issue warning (by site operator) for duration of that week: "Toxic blue-green algae. Risk of skin irritation or intestinal problems." In case of daily site inspection, reevaluate the warning on a daily basis.
	chlorophyll *a*: > 0 to < 12.5 µg/L	surveillance level: continue fortnightly monitoring
	chlorophyll *a*: > 75 µg/L	alert level 2: weekly monitoring and advice against bathing (by public authority): "You are advised not to bathe in this water;" prohibition by local authority is possible.
	chlorophyll *a*: ≥ 12.5 to ≤ 75 µg/L	alert level 1: weekly monitoring and issue warning (by site operator) for duration of that week: "Toxic blue-green algae. Risk of skin irritation or intestinal problems." In case of daily site inspection, reevaluate the warning on a daily basis.
	surface scum: category 1	surveillance level: continue fortnightly monitoring
	surface scum: category 2	alert level 1: weekly monitoring and issue warning (by site operator) for duration of that week: "Toxic blue-green algae. Risk of skin irritation or intestinal problems". In case of daily site inspection, reevaluate the warning on a daily basis.
	surface scum: category 3	alert level 2: weekly monitoring and advice against bathing (by public authority): "You are advised not to bathe in this water"; prohibition by local authority is possible.
New Zealand[h]	cyanobacteria (benthic): 20−50 percent coverage of potentially toxigenic cyanobacteria attached to substrate	alert (amber mode): • Notify the public health unit. • Increase sampling to weekly.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
		• Recommend erecting an information sign. • Consider increasing the number of survey sites. • If toxigenic cyanobacteria dominate the samples, testing for cyanotoxins is advised. If cyanotoxins are detected in mats or water samples, consult the testing laboratory to determine if levels are hazardous.
	cyanobacteria (benthic): greater than 50 percent coverage of potentially toxigenic cyanobacteria attached to substrate	action (red mode) situation 1: • Immediately notify the public health unit • If potentially toxic taxa are present (see Table 2) then consider testing samples for cyanotoxins • Notify the public of the potential risk to health
	cyanobacteria (benthic): Up to 20 percent coverage of potentially toxigenic cyanobacteria attached to substrate	surveillance (green mode): • Undertake fortnightly surveys between spring and autumn at representative locations in the water body where known mat proliferations occur and where there is recreational use
	cyanobacteria (benthic): up to 50 percent where potentially toxigenic cyanobacteria are visibly detaching from the substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as the river level drops.	action (red mode) situation 2: • Immediately notify the public health unit • If potentially toxic taxa are present (see Table 2) then consider testing samples for cyanotoxins. • Notify the public of the potential risk to health
	cyanobacteria (total): < 0.5 mm^3/L (biovolume equivalent of the combined total of all cyanobacteria)	surveillance (green mode): • Undertake weekly or fortnightly visual inspection and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn
	cyanobacteria (total): $\leq$ 500 cells/mL	surveillance (green mode): • Undertake weekly or fortnightly visual inspection and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn
	cyanobacteria (total): $\geq$ 1.8 mm^3/L (biovolume equivalent of potentially toxic cyanobacteria)	action (red mode) situation 1: • Continue monitoring as for alert (amber mode) • If potentially toxic taxa are present (see Table 1), then consider testing samples for cyanotoxins • Notify the public of a potential risk to health
	cyanobacteria (total): $\geq$ 0.5 to < 1.8 mm^3/L (biovolume equivalent of potentially toxic cyanobacteria)	alert (amber mode): • Increase sampling frequency to at least weekly

Jurisdiction	Recreational Water Guideline Level	Recommended Action
		• Notify the public health unit • Multiple sites should be inspected and sampled
	cyanobacteria (total): ≥ 0.5 to < 10 mm^3/L (total biovolume of all cyanobacterial material where the cyanobacterial population has been tested and shown not to contain known toxins)	alert (amber mode): • Increase sampling frequency to at least weekly. • Notify the public health unit. • Multiple sites should be inspected and sampled.
	cyanobacteria (total): ≥ 10 mm^3/L (total biovolume of all cyanobacterial material where the cyanobacterial population has been tested and shown not to contain known toxins)	action (red mode) situation 2: • Continue monitoring as for alert (amber mode) • If potentially toxic taxa are present (see Table 1), then consider testing samples for cyanotoxins • Notify the public of a potential risk to health
	cyanobacterial scums consistently present for more than several days in a row	action (red mode) situation 3: • Continue monitoring as for alert (amber mode) • If potentially toxic taxa are present (see Table 1), then consider testing samples for cyanotoxins • Notify the public of a potential risk to health
	microcystins (total): ≥ 12 µg/L	action (red mode) situation 1: • Continue monitoring as for alert (amber mode) • If potentially toxic taxa are present (see Table 1), then consider testing samples for cyanotoxins • Notify the public of a potential risk to health
Poland[c]	visible blooms	sampling of bathing sites not less than 4 times per season (the interval between sampling does not exceed one month), including responses to cyanobacteria if blooms are observed.
Scotland[e]	chlorophyll *a*: ≥ 10 µg/L with dominance of cyanobacteria	1. watch for scum or conditions conducive to scums. 2. discourage bathing and further investigate hazard. 3. post on-site risk advisory signs. 4. inform relevant authorities.
	cyanobacteria: ≥ 20,000 cells /mL	1. watch for scum or conditions conducive to scums. 2. discourage bathing and further investigate hazard. 3. post on-site risk advisory signs. 4. inform relevant authorities.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	cyanobacterial scum formation in bathing areas	1. immediate action to control contact with scums; possible prohibition of swimming and other water-contact activities. 2. public health follow-up investigation. 3. inform public and relevant authorities.
Singapore[c]	chlorophyll *a*: ≤ 50 µg/L (of 95 percent of a 3-year rolling period)	status of the sites reviewed annually. If the assessment is that the water body is unsuitable for primary water contact activities, the public is notified.
Spain[c]	cyanobacteria proliferation potential (High, Medium, Low)	criteria for assessment of health risk and response are set locally; some health authorities use WHO scheme, others include further risk parameters (such as number of users, type of use); temporary closure has occasionally occurred based on the abundance of cyanobacteria.
Turkey[c]	cells: < 20,000 cells/mL	level 1: recreational activities are allowed to continue and users are informed by posters on site. Monitoring (sampling, counting and species identification) should be done fortnightly.
	cells: 20,000–100,000 cells/mL	level 2: At > 20 000 cells/mL, microcystins are analyzed. If microcystin-LR equivalents >25 µg/L, immediate action to inform relevant authorities and public. Discourage users from swimming and other water-contact activities by advisory signs on site.
	chlorophyll *a* (if dominated by cyanobacteria): < 10 µg/L	level 1: recreational activities are allowed to continue and users are informed by posters on site. Monitoring (sampling, counting and species identification) should be done fortnightly.
	microcystin-LR: < 10 µg/L equivalents	level 1: recreational activities are allowed to continue and users are informed by posters on site. Monitoring (sampling, counting and species identification) should be done fortnightly.
	microcystin-LR: > 25 µg/L equivalents	level 2: At > 20,000 cells/mL, microcystins are analyzed. If microcystin-LR equivalents >25 µg/L, immediate action to inform relevant authorities and public. Discourage users from swimming and other water-contact activities by advisory signs on site.
	visible scum in bathing area	level 3: all activities in the water may be prohibited
World Health Organization (WHO)[b,g]	chlorophyll *a*: 10 µg/L with dominance of cyanobacteria	low risk: post on-site advisory signs, inform relevant authorities
	chlorophyll *a*: 50 µg/L with dominance of cyanobacteria	moderate risk: watch for scums or conditions conducive to scums, discourages swimming and further investigate hazard, post on-site risk advisory signs, inform relevant authorities
	cyanobacteria: 100,000 cells/mL	moderate risk: watch for scums or conditions conducive to scums, discourages swimming and further investigate hazard, post on-site risk advisory signs, inform relevant authorities
	cyanobacteria: 20,000 cells/mL	low risk: post on-site advisory signs, inform relevant authorities

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	cyanobacterial scum formation in areas where whole-body contact and/or risk of ingestions/aspiration occur	high risk: immediate action to control contact with scums, possible prohibition of swimming and other water contact activities, public health follow-up investigation, inform public and relevant authorities

[a] Australian Government National Health and Medical Research Council (2008). Guidelines for Managing Risk in Recreational Water.

[b] Chorus, I. and Bartram, J. (eds.) (1999). Toxic cyanobacteria in water: A guide to public health significance, monitoring and management. E. & F.N. Spon / Chapman & Hall, London, United Kingdom.

[c] Federal Environment Agency (Germany) (2012). Current approaches to Cyanotoxin risk assessment, risk management and regulations in different countries.

[d] Health Canada (2012). Guidelines for Canadian Recreational Water Quality, Third Edition. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H129-15/2012E).

[e] Scottish Government Health and Social Care Directorates Blue-Green Algae Working Group (2012). Cyanobacteria (Blue-Green Algae) in Inland and Inshore Waters: Assessment and Minimization of Risks to Public Health.

[f] European Parliament and the Council of the European Union (2006). Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC.

[g] WHO (World Health Organization) (2003). Guidelines for Safe Recreational Water Environments: Volume 1: Coastal and Fresh Waters. World Health Organization.

[h] Wood, S; Hamilton, D; Safi, K; Williamson, W. (2008). New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters: Interim Guidelines. New Zealand Ministry for the Environment and Ministry of Health.

APPENDIX B. STATE RECREATIONAL WATER GUIDELINES FOR CYANOTOXINS AND CYANOBACTERIA

EPA compiled the information presented in this appendix based on searches of state websites for publicly available information regarding guidelines or action levels for cyanotoxins and cyanobacteria. The website research was completed in November 2015. Subsequent direct personal communication of state guidelines revealed some updates for a few states later in 2015 and in early 2016.

Table B-1. Summary Counts of State Recreational Water Guidelines for Cyanotoxins and Cyanobacteria by Type and Scope of Guidelines

Recreational Water Guideline Type and Scope	Number of States and List of States	Additional Information
Quantitative guidelines for cyanobacteria only	6 states Arizona, Connecticut, Idaho, Maine, New Hampshire, Wisconsin	Measurements for these criteria include cyanobacterial cell densities, proportion of toxigenic cyanobacteria, chlorophyll concentration, and Secchi disk depth measurements.
Quantitative guidelines for cyanotoxins only	7 states California, Colorado, Illinois, Iowa, Nebraska, North Dakota, Ohio	State guidelines address four cyanotoxins in order from most to least common: microcystins (20 states) anatoxin-a (9 states) cylindrospermopsin (7 states) saxitoxin (4 states)
Quantitative guidelines for cyanotoxins and either quantitative or qualitative guidelines for cyanobacteria	14 states Indiana, Kansas, Kentucky, Maryland, Massachusetts, New York, Oklahoma, Oregon, Rhode Island, Texas, Utah, Vermont, Virginia, Washington	
Qualitative guidelines only	6 states Delaware, Florida, Montana, North Carolina, North Dakota, West Virginia	Examples include: presence of surface scum visible discoloration presence of potentially toxic algae

Note: EPA found that Texas and North Carolina published guidelines in the past, but the guidelines are no longer found on their websites.

Table B-2. Summary Counts of State Recreational Water Guidelines for Cyanotoxins and Cyanobacteria by Basis of Guidelines

Recreational Water Guideline Basis of Guideline Category	Number of States and List of States
Based on WHO	6 states Colorado, Indiana, Kentucky, Oklahoma, Utah, Wisconsin
Modified WHO	8 states Illinois, Indiana, Iowa, Kentucky, Oklahoma, Oregon, Utah, Virginia
Jurisdiction-specific (i.e., based on risk assessments or site-specific monitoring information)	8 states California, Colorado, Kansas, Massachusetts, Ohio, Oregon, Vermont, Washington
Based on studies or guidelines other than WHO	3 states Idaho, Indiana, Utah
Qualitative evaluations or narrative criteria application (includes states with insufficient documentation to categorize the source of their guideline)	21 states Arizona, Connecticut, Delaware, Florida, Maine, Maryland, Massachusetts, Montana, Nebraska, New Hampshire, New York, North Carolina, North Dakota, Oregon, Rhode Island, Texas, Utah, Virginia, Washington, West Virginia, Wisconsin

Note: Some states are listed in more than one category because they had more than one guideline (e.g., both cyanobacterial cell and cyanotoxin guidelines), and these guidelines fit into different categories.

Table B-3. State Recreational Water Quality Guideline for Cyanotoxins and Cyanobacteria Sorted by Type

State	Recreational Water Guideline Level	Recommended Action	Reference
States with Guidelines Based on Cyanobacteria Only			
Arizona	blue-green algae (mean value based on a minimum of two sample events within one peak season): 20,000 cells/mL and chlorophyll *a* result (mean value based on a minimum of two sample events within one peak season) in target range	violation of the Narrative Nutrient Standard	Arizona Department of Environmental Quality (2008). Narrative Nutrient Standard Implementation Procedures for Lakes and Reservoirs. http://www.azdeq.gov/environ/water/standards/download/draft_nutrient.pdf. Last Accessed: 08/03/2016.
Connecticut	visual rank category 1: visible material is not likely cyanobacteria or water is generally clear	no action	Connecticut Department of Public Health: Connecticut Energy Environment (2013). Guidance to Local Health Departments For Blue–Green Algae Blooms in Recreational Freshwaters. http://www.ct.gov/deep/lib/deep/water/water_quality_standards/guidance_lhd__bga_blooms_7_2013.pdf. Last Accessed: 08/03/2016.
	visual rank category 2: cyanobacteria present in low numbers; there are visible small accumulations but water is generally clear; OR blue-green algae cells > 20,000 cells/mL and < 100,000 cells/mL	notify Connecticut Department of Public Health (CT DPH), Connecticut Department of Energy and Environmental Protection (CT DEEP)	
	visual rank category 3: cyanobacteria present in high numbers; scums may or may not be present; water is discolored throughout; large areas affected; color assists to rule out sediment and other algae; OR blue-green algae cells > 100,000 cells/mL	update/inform CT DPH & CT DEEP and expand risk communication efforts; POSTED BEACH CLOSURE: if public has beach access, alert water users that a blue-green algae bloom is present; POSTED ADVISORY: at other impacted access points	
Idaho	*Microcystis* or *Planktothrix*: >40,000 cells/mL	public health advisory posting by Public Health District in conjunction with water body operator	IDEQ (Idaho Department of Environmental Quality) (2015). Blue-Green Algae Bloom Response Plan: Final. http://www.epa.illinois.gov/topics/water-quality/monitoring/algal-bloom/2013-program/index . Last Accessed: 08/03/2016.
	sum of all potentially toxigenic taxa: ≥ 100,000 cells/mL	public health advisory posting by Public Health District in conjunction with water body operator	
	visible surface scum that is associated with toxigenic species	public health advisory posting by Public Health District in conjunction with water body management agency	

State	Recreational Water Guideline Level	Recommended Action	Reference
Maine	Secchi disk reading < 2 meters caused by algae	body of water considered impaired, but still safe to swim	Maine Department of Environmental Protection (2013). Reports of Algal Blooms. http://www.maine.gov/dep/water/lakes/repbloom.html. Last Accessed: 08/03/2016.
New Hampshire	cyanobacteria: > 50 percent of total cell counts from toxigenic cyanobacteria	post beach advisory	New Hampshire Department of Environmental Services (2014). Beach Advisories. http://des.nh.gov/organization/divisions/water/wmb/beaches/advisories.htm. Last Accessed: 08/03/2016.
Wisconsin	cyanobacteria: > 100,000 cells/mL	post health advisory and possible beach closure	Wisconsin Department of Natural Resources (2012). Draft Blue-Green Algae Section of 303 (d) Report-7/3/2012: Harmful Algal Blooms. http://dnr.wi.gov/lakes/bluegreenalgae/documents/HarmfulAlgalBloomsvs2.pdf. Last Accessed: 08/03/2016.
	visible scum layer	post health advisory and possible beach closure	Werner M, & Masnado R (2014). Guidance for Local Health Departments: Cyanobacteria and Human Health. http://city.milwaukee.gov/ImageLibrary/Groups/healthAuthors/DCP/PDFs/CyanobacteriaLHD.pdf. Last Accessed: 08/03/2016.
States with Guidelines Based on Cyanotoxin(s) Only			
California	anatoxin-a: 90 µg/L	Unclear	Butler N, Carlisle J, Kaley KB, & Linville R (2012). Toxicological Summary and Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins.
	cylindrospermopsin: 4 µg/L	Unclear	
	microcystins: 0.8 µg/L	Unclear	

State	Recreational Water Guideline Level	Recommended Action	Reference
			http://www.waterboards.ca.gov/water_issues/programs/peer_review/docs/calif_cyanotoxins/cyanotoxins053112.pdf. Last Accessed: 08/03/2016.
Colorado	anatoxin-a: ≥ 7 µg/L	issue toxic algae caution: a. post sign with "caution" language. b. perform routine testing for toxin levels. bi. if test results are below caution thresholds, test at least once per week until algae visually subsides. bii. if test results are above caution thresholds, test at least twice per week until toxin levels are below caution thresholds for two consecutive tests. c. notify drinking water providers and county health department if toxin levels exceed the caution thresholds. d. toxic algae caution ends when there is no visual evidence of algae and toxin levels are non-detectable for two consecutive weeks. di. notify drinking water providers and county health department that bloom has ended. dii. remove "caution" sign.	Colorado Department of Public Health & Environment. Algae bloom risk-management toolkit for recreational waters. https://drive.google.com/file/d/0B0tmPQ67k3NVN2U4VHZBcWxPN0E/view. Last Accessed: 10/21/2016
	cylindrospermopsin: ≥ 7 µg/L	issue toxic algae caution: a. post sign with "caution" language. b. perform routine testing for toxin levels. bi. if test results are below caution thresholds, test at least once per week until algae visually subsides. bii. if test results are above caution thresholds, test at least twice per week until toxin levels are below caution thresholds for two consecutive tests. c. notify drinking water providers and county health department if toxin levels exceed the caution thresholds. d. toxic algae caution ends when there is no visual evidence of algae and toxin levels are non-	

State	Recreational Water Guideline Level	Recommended Action	Reference
		detectable for two consecutive weeks. di. notify drinking water providers and county health department that bloom has ended. dii. remove "caution" sign.	
	microcystin-LR: ≥ 10 µg/L and < 20 µg/L	issue toxic algae caution: a. post sign with "caution" language. b. perform routine testing for toxin levels. bi. if test results are below caution thresholds, test at least once per week until algae visually subsides. bii. if test results are above caution thresholds, test at least twice per week until toxin levels are below caution thresholds for two consecutive tests. c. notify drinking water providers and county health department if toxin levels exceed the caution thresholds. d. toxic algae caution ends when there is no visual evidence of algae and toxin levels are non-detectable for two consecutive weeks. di. notify drinking water providers and county health department that bloom has ended. dii. remove "caution" sign.	
	microcystin-LR: ≥ 20 µg/L	issue toxic algae warning: a. immediately post sign with "warning" language. b. take necessary steps to prevent contact with water in affected area for humans and pets. c. notify drinking water providers and county health department if toxin levels exceed warning thresholds. d. test at least twice per week until toxin levels are below warning thresholds for two consecutive tests. e. posting can be reduced to "caution" language when microcystin test results drop below the warning threshold and no new human illness or pet	

State	Recreational Water Guideline Level	Recommended Action	Reference
		deaths have been reported for two consecutive weeks.	
	saxitoxin: ≥ 4 µg/L	issue toxic algae caution: a. post sign with "caution" language. b. perform routine testing for toxin levels. bi. if test results are below caution thresholds, test at least once per week until algae visually subsides. bii. if test results are above caution thresholds, test at least twice per week until toxin levels are below caution thresholds for two consecutive tests. c. notify drinking water providers and county health department if toxin levels exceed the caution thresholds. d. toxic algae caution ends when there is no visual evidence of algae and toxin levels are non-detectable for two consecutive weeks. di. notify drinking water providers and county health department that bloom has ended. dii. remove "caution" sign.	
	potentially toxic algae are visible	issue toxic algae caution: a. post sign with "caution" language. b. perform routine testing for toxin levels. bi. if test results are below caution thresholds, test at least once per week until algae visually subsides. bii. if test results are above caution thresholds, test at least twice per week until toxin levels are below caution thresholds for two consecutive tests. c. notify drinking water providers and county health department if toxin levels exceed the caution thresholds. d. toxic algae caution ends when there is no visual evidence of algae and toxin levels are non-detectable for two consecutive weeks. di. notify drinking water providers and county	

State	Recreational Water Guideline Level	Recommended Action	Reference
		health department that bloom has ended. dii. remove "caution" sign.	
Illinois	microcystin-LR: > 10 µg/L	appropriate lake management personnel and Illinois EPA staff will be notified; follow-up monitoring by the Illinois EPA may occur as professional judgment dictates and staff, laboratory, and financial resources allow	Illinois Environmental Protection Agency (2015). 2013 Statewide Harmful Algal Bloom Program. http://epa.illinois.gov/topics/water-quality/monitoring/algal-bloom/2013-program/index. Last Accessed: 08/03/2016.
California	anatoxin-a: 90 µg/L	unclear	Butler N, Carlisle J, Kaley KB, & Linville R (2012). Toxicological Summary and Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins. http://www.waterboards.ca.gov/water_issues/programs/peer_review/docs/calif_cyanotoxins/cyanotoxins053112.pdf. Last Accessed: 08/03/2016.
	cylindrospermopsin: 4 µg/L	unclear	
	microcystins: 0.8 µg/L	unclear	
Illinois	microcystin-LR: > 10 µg/L	appropriate lake management personnel and Illinois EPA staff will be notified; follow-up monitoring by the Illinois EPA may occur as professional judgment dictates and staff, laboratory, and financial resources allow	Illinois Environmental Protection Agency (2015). 2013 Statewide Harmful Algal Bloom Program. http://epa.illinois.gov/topics/water-quality/monitoring/algal-bloom/2013-program/index. Last Accessed: 08/03/2016.
Iowa	microcystin: ≥ 20 µg/L	warnings are posted at state park beaches	Iowa Environmental Council (2015). State Park Beach Advisories Report. Updated September 3, 2015. http://www.iaenvironment.org/webres/File/Program%20Publications/2015%20State%20Park%20Beach%20Advisories%20Report.pdf. Last Accessed: 08/03/2016.

State	Recreational Water Guideline Level	Recommended Action	Reference
Nebraska	microcystin: ≥ 20 µg/L	health alert; signs posted advising public to use caution; affected swimming beaches will be closed; boating and other recreational activities will be allowed, but public advised to use caution and avoid prolonged exposure to the water	Nebraska Department of Environmental Quality and Nebraska Department of Health and Human Services: Division of Public Health (2016). Fact Sheet: Precautions and facts regarding toxic algae at Nebraska Lakes. http://deq.ne.gov/NDEQProg.nsf/OnWeb/ENV042607. Last Accessed: 08/03/2016.
North Dakota	blue-green algae bloom is present AND microcystin-LR: < 10 µg/L	issue advisory	North Dakota Department of Health: Division of Water Quality (2016). Blue-green algae advisories and warnings. http://www.ndhealth.gov/WQ/SW/HABs/HABs_Information/Blue-greenLakeListings_20160808.pdf. Last Accessed: 10/18/2016.
	blue-green algae bloom is present over a significant portion of the lake AND microcystin-LR: ≥ 10 µg/L	issue warning	
Ohio	anatoxin-a: 300 µg/L	issue no contact advisory	Kasich JR, Taylor M, Butler CW, Zehringer J, & Hodges R (2016). State of Ohio Harmful Algal Bloom Response Strategy For Recreational Waters. http://epa.ohio.gov/portals/35/hab/HABResponseStrategy.pdf. Last Accessed: 08/03/2016.
	anatoxin-a: 80 µg/L	issue recreational public health advisory	
	cylindrospermopsin: 20 µg/L	issue no contact advisory	
	cylindrospermopsin: 5 µg/L	issue recreational public health advisory	
	microcystin-LR: 20 µg/L	issue no contact advisory	
	microcystin-LR: 6 µg/L	issue recreational public health advisory	
	saxitoxin: 0.8 µg/L	issue recreational public health advisory	
	saxitoxin: 3 µg/L	issue no contact advisory	
Nebraska	microcystin: ≥ 20 µg/L	health alert; signs posted advising public to use caution; affected swimming beaches will be closed; boating and other recreational activities will be	Nebraska Department of Environmental Quality and Nebraska Department of Health and Human Services: Division of Public Health

State	Recreational Water Guideline Level	Recommended Action	Reference
		allowed, but public advised to use caution and avoid prolonged exposure to the water	(2016). Fact Sheet: Precautions and facts regarding toxic algae at Nebraska Lakes. http://deq.ne.gov/NDEQProg.nsf/OnWeb/ENV042607. Last Accessed: 08/03/2016.
Ohio	anatoxin-a: 300 µg/L	issue no contact advisory	Kasich JR, Taylor M, Butler CW, Zehringer J, & Hodges R (2015). State of Ohio Harmful Algal Bloom Response Strategy For Recreational Waters. http://epa.ohio.gov/portals/35/hab/HABResponseStrategy.pdf. Last Accessed: 08/03/2016.
	anatoxin-a: 80 µg/L	issue recreational public health advisory	
	cylindrospermopsin: 20 µg/L	issue no contact advisory	
	cylindrospermopsin: 5 µg/L	issue recreational public health advisory	
	microcystin-LR: 20 µg/L	issue no contact advisory	
	microcystin-LR: 6 µg/L	issue recreational public health advisory	
	saxitoxin: 0.8 µg/L	issue recreational public health advisory	
	saxitoxin: 3 µg/L	issue no contact advisory	
Vermont	anatoxin-a: ≥ 10 µg/L	close recreational beaches	Vermont Department of Health (2015). Cyanobacteria (Blue-green Algae) Guidance for Vermont Communities. http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf. Last Accessed: 08/03/2016.
	cylindrospermopsin: ≥ 10 µg/L	close recreational beaches	
	microcystin-LR (equivalents): ≥ 6 µg/L	close recreational beaches	
	visible known blue-green algae bloom/scum or an unknown, potentially blue-green algae (i.e., not pollen), bloom/scum	close recreational beaches	
Washington	anatoxin-a: 1 µg/L	tier 2: local health posts WARNING sign; local health takes additional site-specific steps; minimum weekly sampling. In addition, if history of high toxicity, or reports of illness, pet death than tier 3: local health posts DANGER sign; lake closed	Hardy J, & Washington State Department of Health (2008). Washington State Recreational Guidance for Microcystins (Provisional) and Anatoxin-a (Interim/Provisional). http://www.doh.wa.gov/Portals/1/Doc

State	Recreational Water Guideline Level	Recommended Action	Reference
			uments/4400/334-177-recguide.pdf. Last Accessed: 08/03/2016.
	bloom is forming or a bloom scum is visible (toxic algae may be present); toxin levels do not exceed thresholds	tier 1: local health posts CAUTION sign; samples taken and sent for toxicity tests; weekly sampling until bloom dissipates	Hardy J, & Washington State Department of Health (2011). Washington State Provisional Recreational Guidance for Cylindrospermopsin and Saxitoxin. http://www.doh.wa.gov/portals/1/documents/4400/332-118-cylindrosax%20report.pdf. Last Accessed: 08/03/2016.
	cylindrospermopsin: 4.5 µg/L	tier 2: local health posts WARNING sign; local health takes additional site-specific steps; minimum weekly sampling. In addition, if history of high toxicity, or reports of illness, pet death than tier 3: local health posts DANGER sign; lake closed.	
	microcystins: 6 µg/L	tier 2: local health posts WARNING sign; local health takes additional site-specific steps; minimum weekly sampling. In addition, if history of high toxicity, or reports of illness, pet death than tier 3: local health posts DANGER sign; lake closed.	Hardy J, & Washington State Department of Health (2008). Washington State Recreational Guidance for Microcystins (Provisional) and Anatoxin-a (Interim/Provisional). http://www.doh.wa.gov/Portals/1/Documents/4400/334-177-recguide.pdf. Last Accessed: 08/03/2016.
Washington (continued)	saxitoxin: 75 µg/L	tier 2: local health posts WARNING sign; local health takes additional site-specific steps; minimum weekly sampling. In addition, if history of high toxicity, or reports of illness, pet death than tier 3: local health posts DANGER sign; lake closed.	Hardy J, & Washington State Department of Health (2011). Washington State Provisional Recreational Guidance for Cylindrospermopsin and Saxitoxin. http://www.doh.wa.gov/portals/1/documents/4400/332-118-cylindrosax%20report.pdf. Last Accessed: 08/03/2016.
States with Guidelines Based on Cyanobacteria and Cyanotoxin(s)			
Indiana	anatoxin-a: 80 µg/L	issue recreation advisory	Indiana Department of Environmental Management (2015). Addressing
	blue-green algae: 100,000 cells/mL	issue recreation advisory	

State	Recreational Water Guideline Level	Recommended Action	Reference
	cylindrospermopsin: 5 µg/L	issue recreation advisory	Concerns About Blue-Green Algae: Indiana Reservoir and Lake Update. http://www.in.gov/idem/algae/2310.htm. Last Accessed: 08/03/2016.
	microcystin-LR: 20 µg/L	close beaches	
	microcystin-LR: 6 µg/L	issue recreation advisory	
Kansas	cyanobacteria: ≥ 10,000,000 cells/mL	recommended that all in-lake recreation cease and that picnic, camping and other public land activities adjacent to affected waters be closed	Kansas Department of Health & Environment (2015). Guidelines for Addressing Harmful Algal Blooms in Kansas Recreational Waters. http://www.kdheks.gov/algae-illness/download/HAB_policy.pdf. Last Accessed: 08/03/2016.
	cyanobacteria: ≥ 250,000 cells/mL	issue public health warning	
	cyanobacteria: ≥ 80,000 and < 250,000 cells/mL	issue public health watch	
	microcystin: ≥ 2,000 µg/L	recommended that all in-lake recreation cease and that picnic, camping and other public land activities adjacent to affected waters be closed	
	microcystin: ≥ 20 µg/L	issue public health warning	
	microcystin: ≥ 4 and < 20 µg/L	issue public health watch	
Kentucky	blue-green algae: > 100,000 cells/mL	issue an harmful algal bloom (HAB) advisory	Kentucky Department for Environmental Protection (2014). Harmful Algal Blooms: Background. http://water.ky.gov/waterquality/Documents/HAB_FACTs/HAB%20Background%20Fact%20Sheet.pdf. Last Accessed: 08/03/2016.
	microcystins: > 20 µg/L	issue recreational use advisory	Commonwealth of Kentucky: Department for Environmental Protection Division of Water (2015). Harmful Algal Blooms. http://water.ky.gov/waterquality/pages/HABS.aspx. Last Accessed: 08/03/2016.

State	Recreational Water Guideline Level	Recommended Action	Reference
Maryland	*Microcystis aeruginosa* or other potential microcystin producing blue-green algae > 40,000 cells/mL, and samples contain microcystins: > 10 ppb	put up signs advising public of health risk, notify local press (through joint DHMH, DNR, MDE press release) and coordinate with local health department, place advisory information on DNR web site (Eyes on the Bay), Maryland Healthy Beaches web site if a swimming beach is affected, or other local web site. MDE will initiate emergency closure to shellfish harvesting if warranted, and coordinate with DNR Natural Resource Police	Maryland Department of Natural Resources (2010). Harmful Algal Bloom (HAB) Monitoring and Management SOP. SOP document (with edits) sent via email correspondence with Catherine Wazniak, Program Manager at the MD DNR, on February 22, 2016.
	presence of potentially toxic algae	issue algae bloom beach alert	Maryland Department of Natural Resources (2010). Harmful Algal Bloom (HAB) Monitoring and Management SOP. SOP document (with edits) sent via email correspondence with Catherine Wazniak, Program Manager at the MD DNR, on February 22, 2016.
Massachusetts	blue-green algae: > 50,000 cells/mL	toxin testing of lysed cells should be done to ensure that guideline of 14 ppb is not exceeded	Massachusetts Bureau of Environmental Health (2015). MDPH Guidelines for Cyanobacteria in Freshwater Recreational Water Bodies in Massachusetts. Boston, Massachusetts. http://www.mass.gov/eohhs/docs/dph/environmental/exposure/protocol-cyanobacteria.pdf. Last Accessed: 08/03/2016.
	blue-green algae: > 70,000 cells/mL	post an advisory against contact with the water	
	microcystins: > 14 µg/L	post an advisory against contact with the water	
	visible cyanobacteria scum or mat is evident	MDPH recommends an immediate posting by the local health department, state agency, or relevant authority to advise against contact with the water body	
New York	visible HAB	prohibit wading, swimming, diving and any water contact activities in the swimming area; post beach closure and advisory signs at the beach and other shoreline access areas; contact local health department	June, Stephanie. Senior Sanitarian at the New York State Department of Health. Email correspondence on Feb. 23, 2016.

State	Recreational Water Guideline Level	Recommended Action	Reference
	microcystins: 10 µg/L	prohibit wading, swimming, diving and any water contact activities in the swimming area; post beach closure and advisory signs at the beach and other shoreline access areas; contact local health department; to reopen swim areas: • water must be visibly clear of HABs or associated material for one day • at that time, a water sample is to be collected and tested for microcystins • if the sample indicates toxin levels <10 µg/L and the HAB has not returned to the swim area, the signs may be removed and the beach may be reopened	June, Stephanie. Senior Sanitarian at the New York State Department of Health. Email correspondence on Feb. 23, 2016.
	bloom: credible report or digital imagery of a bloom determined as likely to be potentially toxic cyanobacteria by the Department of Environmental Conservation (DEC) or Department of Health (DOH) staff; a descriptive field report from professional staff or trained volunteer may be used as a report in absence of digital images; for all other surveillance reports received from the general public, lay monitors, etc., DEC HABs Program staff will determine if a bloom is suspicious and whether collection of a sample is feasible or warranted	post DEC blue-green algal bloom notice: suspicious bloom	Gorney, Rebecca. Research Scientist at New York State Department of Environmental Conservation. Email correspondence on Feb. 23, 2016.
	blue green chlorophyll *a*: >25-30 µg/L	post DEC blue-green algal bloom notice: confirmed bloom	Gorney, Rebecca. Research Scientist at New York State Department of Environmental Conservation. Email correspondence on Feb. 23, 2016.
	potential toxin-producing cyanobacteria taxa: >50% of algae present	post DEC blue-green algal bloom notice: confirmed bloom	Gorney, Rebecca. Research Scientist at New York State Department of Environmental Conservation. Email correspondence on Feb. 23, 2016.

State	Recreational Water Guideline Level	Recommended Action	Reference
	microcystin-LR: 4 µg/L	post DEC blue-green algal bloom notice: confirmed bloom	Gorney, Rebecca. Research Scientist at New York State Department of Environmental Conservation. Email correspondence on Feb. 23, 2016.
	anatoxin-a or other cyanotoxins: high risk of exposure based on consult among DEC or DOH staff	post DEC blue-green algal bloom notice: confirmed bloom	Gorney, Rebecca. Research Scientist at New York State Department of Environmental Conservation. Email correspondence on Feb. 23, 2016.
	microcystin-LR: 10 µg/L in open water sample	post DEC blue-green algal bloom notice: confirmed with high toxins	Gorney, Rebecca. Research Scientist at New York State Department of Environmental Conservation. Email correspondence on Feb. 23, 2016.
	microcystin-LR: 20 µg/L in shoreline sample	post DEC blue-green algal bloom notice: confirmed with high toxins	New York State Department of Environmental Conservation. Water Clarity Fact Sheet. http://www.dec.ny.gov/docs/water_pdf/cslaplkpara.pdf. Last Accessed: 10/23/2015.
Oklahoma	cyanobacteria: 100,000 cell/mL	issue advisory	Oklahoma Legislature (2012). SB 259 Bill Summary. http://webserver1.lsb.state.ok.us/CF/2011-12%20SUPPORT%20DOCUMENTS/BILLSUM/House/SB259%20ccr%20a%20billsum.doc. Last Accessed: 08/03/2016.
	microcystin: > 20 µg/L	issue advisory	
Oregon	anatoxin-a: ≥ 20 µg/L	issue public health advisory	Oregon Health Authority (2016). Oregon Harmful Algae Bloom Surveillance (HABS) Program Public Health Advisory Guidelines: Harmful Algae Blooms in Freshwater Bodies. https://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulA
	cylindrospermopsin: ≥ 20 µg/L	issue public health advisory	
	microcystin: ≥ 10 µg/L	issue public health advisory	
	Microcystis: > 40,000 cells/mL	issue public health advisory	
	Planktothrix: > 40,000 cells/mL	issue public health advisory	

State	Recreational Water Guideline Level	Recommended Action	Reference
	saxitoxin: ≥ 10 µg/L	issue public health advisory	lgaeBlooms/Documents/HABPublicHealthAdvisoryGuidelines.pdf. Last Accessed: 08/03/2016.
	toxigenic species: > 100,000 cells/mL	issue public health advisory	
	visible scum with documentation and testing	issue public health advisory	
Rhode Island	cyanobacteria: > 70,000 cells/mL	issue health advisory	Rhode Island Department of Environmental Management, & Rhode Island Department of Health (2013). Cyanobacteria Related Public Health Advisories in Rhode Island. http://www.health.ri.gov/publications/datareports/2013CyanobacteriaBloomsInRhodeIsland.pdf. Last Accessed: 08/03/2016.
	microcystin-LR: ≥ 14 µg/L	issue health advisory	
	visible cyanobacteria scum or mat	issue health advisory	
Texas	>100,000 cell/mL of cyanobacterial cell counts and >20µg/L microcystin	blue-green algae awareness level advisory	U.S. EPA (United States Environmental Protection Agency) (2016). What are the Standards or Guidelines for Cyanobacteria/Cyanotoxin in Recreational Water. https://www.epa.gov/nutrient-policy-data/guidelines-and-recommendations#what3. Last Accessed: 08/03/2016.
Utah	anatoxin-a: > 20 µg/L	issue caution advisory; post CAUTION sign; weekly sampling recommended	Utah Department of Environmental Quality and Department of Health (2015). Utah Guidance for Local Health Departments: Harmful Algal Blooms and Human Health. http://www.deq.utah.gov/Topics/Water/HealthAdvisoryPanel/docs/07Jul/HarmfulAlgalBlooms.pdf. Last Accessed: 08/03/2016.
	blue-green algae: >10,000,000 cells/mL	issue danger advisory; post DANGER sign; weekly sampling recommended; consider closure	
	blue-green algae: 100,000-10,000,000 cells/mL	issue warning advisory; post WARNING sign; weekly sampling recommended	
	blue-green algae: 20,000-100,000 cells/mL	issue caution advisory; post CAUTION sign; weekly sampling recommended	

State	Recreational Water Guideline Level	Recommended Action	Reference
	large scum mat layer	issue danger advisory; post DANGER sign; weekly sampling recommended; consider closure	
	microcystin: > 2,000 µg/L	issue danger advisory; post DANGER sign; weekly sampling recommended; consider closure	
	microcystin: 20-2,000 µg/L	issue warning advisory; post WARNING sign; weekly sampling recommended	
	microcystin: 4-20 µg/L	issue caution advisory; post CAUTION sign; weekly sampling recommended	
	reports of animal illnesses or death	issue warning advisory; post WARNING sign; weekly sampling recommended	
	reports of human illness	issue danger advisory; post DANGER sign; weekly sampling recommended; consider closure	
Virginia	blue-green algal "scum" or "mats" on water surface	immediate public notification to avoid all recreational water contact where bloom is present; continue weekly sampling	Virginia Department of Health (Division of Environmental Epidemiology) (2012). Virginia Recreational Water Guidance for Microcystin and *Microcystis* Blooms: Provisional Guidance. https://www.vdh.virginia.gov/epidemiology/dee/HABS/documents/VDHMicrocystisGuidance.pdf. Last Accessed: 08/03/2016.
	microcystin: > 6 µg/L	immediate public notification to avoid all recreational water contact where bloom is present; continue weekly sampling	
	Microcystis: > 100,000 cells /mL	immediate public notification to avoid all recreational water contact where bloom is present; continue weekly sampling	
	Microcystis: 20,000 to 100,000 cells/mL	notify public through press release and/or signage; advise people and pet-owners that harmful algae are present; initiate weekly water sampling	
	Microcystis: 5,000 to < 20,000 cells/mL	local agency notification; initiate bi-weekly water sampling	
States with Qualitative Guidelines Only			
Delaware	thick green, white, or red scum on surface of pond	post water advisory signs	Delaware Department of Natural Resources and Environmental Control:

State	Recreational Water Guideline Level	Recommended Action	Reference
			Division of Water. Blue-Green Algae in Delaware. http://www.dnrec.delaware.gov/wr/INFORMATION/OTHERINFO/Pages/Blue-GreenAlgae.aspx. Last Accessed: 08/03/2016.
Florida	cyanobacteria bloom	health advisory	Florida Department of Environmental Protection (2016). South Florida Algal Bloom Monitoring and Response. https://depnewsroom.wordpress.com/south-florida-algal-bloom-monitoring-and-response/. Last Accessed: 08/16/2016. Florida Department of Health (2016). Blue-Green Algae (Cyanobacteria). http://www.floridahealth.gov/environmental-health/aquatic-toxins/cyanobacteria.html. Last Accessed: 08/16/2016.
Montana	reservoirs that seem stagnated and harbor large quantities of algae	the Montana Department of Environmental Quality advises people to avoid swimming in ponds, lakes, or reservoirs	State of Montana Newsroom (2015). DEQ Issues Advisory on Blue-Green Algae Blooms: Ponds, Lakes, and Reservoirs Most Often Affected. http://news.mt.gov/Home/ArtMID/24469/ArticleID/1564/DEQ-Issues-Advisory-on-Blue-Green-Algae-Blooms. Last Accessed: 08/03/2016.
North Carolina	visible discoloration or surface scum	Microcystin testing	North Carolina Health and Human Services: Division of Public Health (2014). Occupational & Environmental Epidemiology: Cyanobacteria (Blue-green Algae). http://epi.publichealth.nc.gov/oee/a_z/

State	Recreational Water Guideline Level	Recommended Action	Reference
			algae.html. Last Accessed: 08/03/2016.
West Virginia	blue-green algal blooms observed and monitored	issue public health advisory	West Virginia Department of Health & Human Resources (2015). DHHR Continuing to Monitor Blue-Green Algal Blooms on the Ohio River: Residents Advised to Adhere to Public Health Advisory. http://www.dhhr.wv.gov/News/2015/Pages/DHHR-Continuing-to-Monitor-Blue-Green-Algal-Blooms-on-the-Ohio-River%3B-Residents-Advised-to-Adhere-to-Public-Health-Advisory.aspx. Last Accessed: 08/03/2016.

APPENDIX C. LITERATURE SEARCH DOCUMENTATION

The recreational ambient water quality criteria document for microcystins, cylindrospermopsin, and cyanobacteria relied significantly on information identified, reviewed, and synthesized in U.S. EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins, Heath Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin, Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins*, and *Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin* ((U.S. EPA (2015c); U.S. EPA (2015d)); (U.S. EPA 2015a; U.S. EPA 2015b). EPA conducted supplemental literature searches to answer additional questions related to recreational exposures, exposure factors, and to identify new health data.

For the Health Effects Support Documents, EPA conducted a comprehensive literature search from January 2013 to May 2014 using Toxicology Literature Online (TOXLINE), PubMed, and Google Scholar. EPA assembled available information on occurrence; environmental fate; mechanisms of toxicity; acute, short-term, subchronic, and chronic toxicity and cancer in humans and animals; and toxicokinetics and exposure. For a detailed description of the literature review search and strategy, see the Health Effects Support Documents for microcystins and cylindrospermopsin (U.S. EPA 2015c; U.S. EPA 2015d).

EPA conducted supplemental literature searches in September 2015 to capture references published since the completion of the Health Effects Support Documents literature searches and to account for the recreational exposure scenario. The specific questions investigated include:

1. What levels of anatoxin-a, cylindrospermopsin, or microcystins are humans—of all ages, including children—exposed to through recreational use (activities) in freshwaters or marine waters from incidental ingestion, inhalation, and dermal exposure routes?
2. What health effects information for humans or animals exposed to cylindrospermopsin or microcystins (through ingestion, inhalation, and dermal exposure routes) has been published since the health effects literature searches were conducted for EPA's 2015 Health Effects Support Documents for cylindrospermopsin and microcystins?
3. What recreational water use safety levels or criteria have been set for microcystins or cylindrospermopsin by states or international governments, and how did they derive them?
4. What new information, if any, is available regarding how aquatic recreational exposure ingestion rates in children differ among age groups between 0 and 18 years?
5. What incidents of companion animal (e.g., dogs, horses) or livestock poisonings, including mortality or adverse health effects, due to exposure to cyanotoxins in freshwaters, marine waters, or beaches have occurred in the past 15 years? Specifically, when and where did these incidents occur, to which cyanotoxin were the animals exposed, how were they exposed, and what were the weights and breeds of the affected animal(s)?

EPA implemented a unique literature search strategy to address each research question. Trial searches were conducted, and results were evaluated to refine the search strategies (e.g., to reduce retrieval of citations unrelated to the research questions). The search strings were refined to improve the relevancy of the results. The literature search strategies implemented for each research question are subsequently detailed.

Research Question 1: What levels of anatoxin-a, cylindrospermopsin, or microcystins are humans—of all ages, including children—exposed to through recreational use (activities) in freshwaters or marine waters, from incidental ingestion, inhalation, and dermal exposure routes?

EPA searched the bibliographic databases, PubMed and Web of Science (WoS), to identify candidate journal article literature relevant to human exposure to anatoxin-a, cylindrospermopsin, or microcystins through recreational activities. PubMed and WoS contain peer-reviewed journal abstracts and articles on various biological, medical, public health, and chemical topics. The WoS search string differs slightly from the PubMed search string due to how the search engines treat search terms with more than one word. Both search strings are presented below.

Results

The searches returned 321 journal articles after removing duplicates between PubMed and WoS results. Based on a screening review of each article's title and abstract, EPA retrieved 9 articles that appeared to be studies that measured, reviewed, or estimated human recreational exposure to cyanotoxins.

PubMed Search:

("*A. lemmermannii Raphidiopsis mediterranea*" OR *Anabaena flos-aquae* OR *flos-aquae* OR anatoxin-a OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia*)

AND

("boogie board" OR "boogie boarding" OR "jet ski" OR "jet skier" OR "jet skiers" OR "jet skiing" OR "water ski" OR "water skier" OR "water skiers" OR "water skiing" OR aerosol OR boat OR boating OR boats OR bodyboard OR bodyboarding OR canoe OR canoeing OR canoes OR capsize OR capsized OR dermal OR inhalation OR inhale OR kayak OR kayaker OR kayaking OR kayaks OR kneeboard OR kneeboarding OR paddle OR paddling OR raft OR rafting OR rafts OR recreation OR recreational OR rowing OR skin OR surf OR surfer OR surfing OR swim OR swimmer OR swimmers OR swimming OR tubing OR wading OR wakeboarding OR wakeboard)

AND

("marine water" OR "surface water" OR beach OR beaches OR estuaries OR estuarine OR estuary OR "fresh water" OR freshwater OR lake OR lakes OR ocean OR oceans OR pond OR ponds OR reservoir OR reservoirs OR river OR rivers OR sea OR stream OR streams OR water)

Filters: English
Date search was conducted: 10/9/2015
Publication dates searched: 1/1/1995 – 10/9/2015

Web of Science Search:

("*lemmermannii Raphidiopsis mediterranea*" OR *Anabaena flos-aquae* OR *flos-aquae* OR anatoxin OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia*)

AND

("boogie board" OR "boogie boarding" OR "jet ski" OR "jet skier" OR "jet skiers" OR "jet skiing" OR "water ski" OR "water skier" OR "water skiers" OR "water skiing" OR aerosol OR boat OR boating OR boats OR bodyboard OR bodyboarding OR canoe OR canoeing OR canoes OR capsize OR capsized OR dermal OR inhalation OR inhale OR kayak OR kayaker OR kayaking OR kayaks OR kneeboard OR kneeboarding OR paddle OR paddling OR raft OR rafting OR rafts OR recreation OR recreational OR rowing OR skin OR surf OR surfer OR surfing OR swim OR swimmer OR swimmers OR swimming OR tubing OR wading OR wakeboarding OR wakeboard)

AND

("marine water" OR "surface water" OR beach OR Beaches OR estuaries OR estuarine OR estuary OR "fresh water" OR freshwater OR lake OR lakes OR ocean OR oceans OR pond OR ponds OR reservoir OR reservoirs OR river OR rivers OR sea OR stream OR streams OR water)

Filters: English
Date search was conducted: 10/9/2015
Publication dates searched: 1/1/1995–10/9/2015

Research Question 2: What health effects information for humans or animals exposed to microcystins, cylindrospermopsin, or anatoxin-a (through ingestion, inhalation, and dermal exposure routes) has been published since the health effects literature searches were conducted for EPA's 2015 *Health Effects Support Documents for Cylindrospermopsin and Microcystins*?

EPA searched PubMed and WoS to identify candidate journal article literature relevant to health effects associated with exposure to anatoxin-a, cylindrospermopsin, or microcystins. The WoS search string differs slightly from the PubMed search string due to how the search engines treat search terms with more than one word. Both search strings are presented below.

Results

The searches returned 1,000 journal articles after removing duplicates between PubMed and WoS results. Based on a screening review of each article's title and abstract, EPA retrieved 40 articles that appeared to be prospective human epidemiological studies (n = 1), ecological human epidemiologic studies (n = 2), reviews of human health effects (n = 4), *in vivo* animal studies (n = 30), or reviews of *in vivo* animal studies (n = 3).

PubMed Search:

("*A. lemmermannii Raphidiopsis mediterranea*" OR *Anabaena flos-aquae* OR *flos-aquae* OR anatoxin-a OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia*)

AND

("non cancer" OR "blurred vision" OR "cell damage" OR "cellular damage" OR "health effect" OR "health endpoint" OR "health outcome" OR "health risk" OR "loss of protein" OR "loss of water" OR "micronucleated binucleate cell" OR abdominal pain OR ache OR acute OR alanine aminotransferase OR allergic OR allergies OR allergy OR aspartate aminotransferase OR blister OR blistered OR blisters OR cancer OR carcinogen OR carcinogenic OR carcinogens OR chronic OR clinical OR cough OR dermal OR detoxification OR detoxify OR develop OR development OR developmental OR dialysis OR diarrhea OR disease OR DNA OR dyspnea OR electrolyte OR emergency room OR enzyme OR enzymes OR epidemiologic OR epidemiological OR epidemiology OR epilepsy OR epileptic OR epithelium OR eye OR failure OR fever OR gastrointestinal OR genetox OR genotoxic OR glutamyltransferase OR head OR hematologic OR hematological OR hepatic OR histopathologic OR histopathological OR histpathology OR hospital OR hospitalizations OR hospitals OR hospitalization OR ill OR illness OR illnesses OR intoxicate OR intoxicated OR irritate OR irritated OR kidney OR larynx OR lesion OR lesions OR liver OR lung OR lymph OR lymph nodes OR lymphatic OR metabolic OR metabolism OR mucosa OR mutate OR mutated OR mutation OR mutations OR nausea OR necrosis OR neonatal OR neonate OR neonates OR neoplasm OR neurologic OR neurological OR noncancer OR oral OR organ OR pain OR placenta OR pneumonia OR polymorphism OR polymorphisms OR prenatal OR red blood cell OR renal OR reproduction OR respiratory OR seizure OR sick OR sickness OR skin OR stomach OR subacute OR subchronic OR symptom OR symptoms OR teratogen OR teratogenic OR teratogens OR throat OR toxic OR toxicity OR trachea OR tumor OR tumors OR urinary OR urine OR vomit OR vomiting OR conjugate OR conjugated OR diagnose OR diagnosis OR diagnosed OR diagnoses)

Filters: English
Date search was conducted: 10/9/2015
Publication dates searched: 1/1/2014–10/9/2015

Web of Science Search:

("*lemmermannii Raphidiopsis mediterranea*" OR *Anabaena flos-aquae* OR *flos-aquae* OR anatoxin OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia*)

AND

("non cancer" OR "blurred vision" OR "cell damage" OR "cellular damage" OR "health effect" OR "health endpoint" OR "health outcome" OR "health risk" OR "micronucleated binucleate cell" OR abdominal pain OR ache OR acute OR alanine aminotransferase OR allergic OR allergies OR allergy OR aspartate aminotransferase OR blister OR blistered OR blisters OR cancer OR carcinogen OR carcinogenic OR carcinogens OR chronic OR clinical OR cough OR

dermal OR detoxification OR detoxify OR develop OR development OR developmental OR dialysis OR diarrhea OR disease OR DNA OR dyspnea OR electrolyte OR emergency room OR enzyme OR enzymes OR epidemiologic OR epidemiological OR epidemiology OR epilepsy OR epileptic OR epithelium OR eye OR failure OR fever OR gastrointestinal OR genetox OR genotoxic OR glutamyltransferase OR head OR hematologic OR hematological OR hepatic OR histopathologic OR histopathological OR histpathology OR hospital OR hospitalizations OR hospitals OR hospitalization OR ill OR illness OR illnesses OR intoxicate OR intoxicated OR irritate OR irritated OR kidney OR larynx OR lesion OR lesions OR liver OR lung OR lymph OR lymph nodes OR lymphatic OR metabolic OR metabolism OR mucosa OR mutate OR mutated OR mutation OR mutations OR nausea OR necrosis OR neonatal OR neonate OR neonates OR neoplasm OR neurologic OR neurological OR noncancer OR oral OR organ OR pain OR placenta OR pneumonia OR polymorphism OR polymorphisms OR prenatal OR red blood cell OR renal OR reproduction OR respiratory OR seizure OR sick OR sickness OR skin OR stomach OR subacute OR subchronic OR symptom OR symptoms OR teratogen OR teratogenic OR teratogens OR throat OR toxic OR toxicity OR trachea OR tumor OR tumors OR urinary OR urine OR vomit OR vomiting OR conjugate OR conjugated OR diagnose OR diagnosis OR diagnosed OR diagnoses)

Filters: English
Date search was conducted: 10/9/2015
Publication dates searched: 1/1/2014–10/9/2015

WoS research areas searched: Environmental Sciences Ecology OR Marine Freshwater Biology OR Toxicology OR Pharmacology Pharmacy OR Public Environmental Occupational Health OR Microbiology OR Immunology OR Biotechnology Applied Microbiology OR Biochemistry Molecular Biology OR Research Experimental Medicine OR Water Resources OR Infectious Disease OR Science Technology Other Topics OR Life Sciences Biomedicine Other Topics OR Gastroenterology Hepatology OR Pediatrics.

Research Question 3: What recreational water use safety levels or criteria have been set for microcystins or cylindrospermopsin by states or international governments and how did they derive them?

To identify state-level recreational guidelines for cyanobacteria and cyanotoxins, EPA searched the websites of state-level departments of public health, environmental health, and natural resources for all 50 U.S. states. If relevant recreational guidelines were not found by searching state-level websites, EPA conducted Google searches of the internet using state names, key terms for cyanobacteria and cyanotoxins (e.g., harmful algal bloom, blue green algae, microcystin, cylindrospermopsin), and key terms for guidelines (e.g., advisory, guidance, guideline, standard, regulation). For international governments, EPA used the 2012 report, *Current Approaches to Cyanotoxin Risk Assessment, Risk Management and Regulations in Different Countries*, by Dr. Ingrid Chorus, Federal Environment Agency, Germany, to identify international government recreational safety levels for cyanobacteria and cyanotoxins. In addition, EPA implemented the same search strategy as used for U.S. states to identify updated international recreational guidelines or guideline levels not featured in the 2012 report by Dr. Ingrid Chorus.

Research Question 4: What new information, if any, is available regarding how aquatic recreational exposure ingestion rates in children differ among age groups between 0 and 18 years?

Search of Bibliographic Databases

EPA searched PubMed, WoS, and Google Scholar to identify literature that has cited, or is similar (based on terms identified in the titles and abstracts) to, the studies that provide water ingestion data for swimmers or during water recreational activities in EPA's (2011) *Exposure Factors Handbook* (i.e.,(Dorevitch et al. 2011; Dufour et al. (2006); Schets et al. 2011). The PubMed and WoS searches were conducted on 10/9/2015, the publication dates searched were 1/1/2011 to 10/9/2015, and an English filter was applied. The Google Scholar search was conducted on 10/9/2015 and could not be limited by year or language.

Results

Together all three searches returned 341 journal articles. Duplicates were removed between PubMed and Wos, but this total might include duplicates between Google Scholar results and WoS/PubMed results. Based on a screening review of each article's title and abstract, EPA retrieved 5 articles, 4 of which were published between 2013–2015 and appeared to measure or estimate incidental water ingestion. EPA also retrieved one 2012 study that assessed duration of non-swimming recreational water exposure by using novel time lapse photography technology.

Google Search of Internet:

In addition, EPA conducted a Google search of the internet focused on specified URL domains (listed in Table C-1) to identify candidate gray literature (e.g., state, federal, or international government reports or guidance). The Google search string is presented below. The Google search of the internet could not be limited by year or language.

Table C-1. Internet URL Domains Searched for Research Question 4

Organization	URL Domain
U.S. Government	.gov .us
All U.S. States	Google Custom Search Engine
Centers for Disease Control and Prevention, including Agency for Toxic Substances and Disease Registry	cdc.gov
Australia, including Australian Department of Health	gov.au
Canada, including Health Canada	gc.ca
European Union, including • European Chemicals Agency • European Commissions on Environment, Public Health, Food, and Health and Consumers	europa.eu
Public Health England	hpa.org.uk
United Kingdom	gov.uk

Organization	URL Domain
Germany	.de
Education websites	.edu
HERA (Human and Environmental Risk Assessment) Project	heraproject.com
World Health Organization	who.int

Results:

The Google search returned 390 results after removing duplicates. Based on a preliminary screen of each result, EPA retrieved two documents which appeared to either derive or cited an incidental ingestion rate while recreating which had not previously been identified during the literature search process.

Google Search of Internet (conducted separately for each URL domain listed in Table C-1)

(pool OR swim OR swimmer OR swimmers OR swimming OR recreation OR recreational)

AND

(adolescents OR boys OR child OR children OR girls OR kids OR teenagers)

AND

(“activity-related ingestion” OR “incidental ingestion” OR “activity-related ingestion” OR “ingestion of water” OR “water ingestion”)

AND

rate

AND

inurl:.

Filters: None
Date search was conducted: 10/9/2015
Dates searched: Not specified
Web browser: Internet Explorer

Research Question 5: What incidents of companion animal (e.g., dogs, horses) or livestock poisonings, including mortality or adverse health effects, due to exposure to cyanotoxins in freshwaters, marine water, or beaches have occurred in the past 15 years? Specifically, when and where did these incidents occur, to which cyanotoxin were the animals exposed, how were they exposed, and what were the weights and breeds of the affected animal(s)?

EPA searched PubMed, WoS, and Agricola to identify candidate journal article literature relevant to companion animal or livestock poisoning due to exposures to cyanobacterial cells, anatoxin-a, cylindrospermopsin, or microcystins. EPA first searched PubMed and WoS with a focus on dogs. EPA conducted two additional searches in PubMed, WoS, and Agricola focused on livestock, and on cats and birds. The search strings for each search iteration are presented below.

Results

The number of journal articles returned by the three searches is provided in Table C-2. Based on a screening review of the article's title and abstract, EPA retrieved 5 of the 35 journal articles retrieved during the search focused on dogs. These 5 articles appeared to provide information about an incident of cyanotoxin exposure to an animal where the authors confirm that the animal was exposed to a cyanotoxin by either measuring the concentration of cyanotoxin found in the animal and/or by sampling the body of water to which the animal had contact.

Table C-2. Number of Journal Articles Returned by Three Search Strategies for Research Question 5

Search Strategy Focus	Number of Results Returned from PubMed, WoS, and Agricola Searches
Dogs	35[a]
Livestock	100
Cats and birds	169[b]

[a] Search conducted in PubMed and WoS only.

[b] Duplicates between PubMed/WoS results and Agricola results were not removed. Therefore, the cats and birds search might include duplicates between Agricola results and PubMed/WoS results.

C.1 Search strategy focused on dogs

PubMed Search:

("*A. lemmermannii Raphidiopsis mediterranea*" OR *flos-aquae* OR anatoxin-a OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia* OR Cyanobacteria OR cyanotoxin OR Cyanotoxins OR "harmful algae" OR "harmful algal bloom" OR blue green algae)

AND

("health effect" OR "health endpoint" OR "health outcome" OR dead OR death OR deaths OR died OR disease OR diseased OR diseases OR exposed OR exposure OR ill OR illness OR illnesses OR infect OR infected OR infection OR infections OR morbidity OR mortality OR poison OR poisoned OR poisoning OR poisonings OR sick OR sickness OR toxic OR toxicity OR diagnose OR diagnosis OR diagnosed OR diagnoses)

AND

(canine OR canines OR dog OR dogs OR "*Canis lupus familiaris*" OR "*Canis familiaris*")

Filters: English
Date search was conducted: 10/5/2015
Publication dates searched: 1/1/2012–10/5/2015

Web of Science Search:

(“*lemmermannii Raphidiopsis mediterranea*” OR *flos-aquae* OR anatoxin OR *Aphanizomenon* OR cylindrospermopsin OR “*C. raciborskii*” OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR “*Cylindrospermopsis raciborskii*” OR *Dolichospermum* OR “*M. aeruginosa*” OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia* OR Cyanobacteria OR cyanotoxin OR Cyanotoxins OR “harmful algae” OR “harmful algal bloom” OR blue green algae)

AND

(“health effect” OR “health endpoint” OR “health outcome” OR dead OR death OR deaths OR died OR disease OR diseased OR diseases OR exposed OR exposure OR ill OR illness OR illnesses OR infect OR infected OR infection OR infections OR morbidity OR mortality OR poison OR poisoned OR poisoning OR poisonings OR sick OR sickness OR toxic OR toxicity OR diagnose OR diagnosis OR diagnosed OR diagnoses)

AND

(canine OR canines OR dog OR dogs OR “*Canis lupus familiaris*” OR “*Canis familiaris*”)

Filters: English
Date search was conducted: 10/5/2015
Publication dates searched: 1/1/2012–10/5/2015

C.2 Search strategy focused on livestock

PubMed and Agricola Searches:

(“*A. lemmermannii Raphidiopsis mediterranea*” OR *flos-aquae* OR anatoxin-a OR *Aphanizomenon* OR cylindrospermopsin OR “*C. raciborskii*” OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR “*Cylindrospermopsis raciborski*i” OR *Dolichospermum* OR “*M. aeruginosa*” OR *Microcystis* OR microcystin OR microcystins OR *Oscillatori*a OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia* OR *Cyanobacteria* OR *cyanotoxin* OR *Cyanotoxins* OR “harmful algae” OR “harmful algal bloom” OR blue green algae)

AND

(“health effect” OR “health endpoint” OR “health outcome” OR dead OR death OR deaths OR died OR disease OR diseased OR diseases OR exposed OR exposure OR ill OR illness OR illnesses OR infect OR infected OR infection OR infections OR morbidity OR mortality OR poison OR poisoned OR poisoning OR poisonings OR sick OR sickness OR toxic OR toxicity OR diagnose OR diagnosis OR diagnosed OR diagnoses)

AND

(alpaca OR alpacas OR bronco OR broncos OR buffalo OR bull OR bulls OR cattle OR colt OR colts OR cow OR cows OR bovine OR bison OR oxen OR donkey OR donkeys OR duck OR ducks OR equine OR ewe OR ewes OR fillies OR filly OR foal OR foals OR gelding OR geldings OR heifer OR heifers OR horse OR horses OR lamb OR lambs OR livestock OR llama OR llamas OR mare OR mares OR mule OR mules OR mustang OR mustangs OR ponies OR pony OR ram OR rams OR sheep OR stallion OR stallions OR steer OR pig OR pigs OR piglet OR piglets)

Filters: English
Date search was conducted: 11/25/2015
Publication dates searched: 1/1/2012–11/25/2015

Web of Science Search:

("*lemmermannii Raphidiopsis mediterranea*" OR *flos-aquae* OR anatoxin OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia* OR Cyanobacteria OR cyanotoxin OR Cyanotoxins OR "harmful algae" OR "harmful algal bloom" OR blue green algae)

AND

("health effect" OR "health endpoint" OR "health outcome" OR dead OR death OR deaths OR died OR disease OR diseased OR diseases OR exposed OR exposure OR ill OR illness OR illnesses OR infect OR infected OR infection OR infections OR morbidity OR mortality OR poison OR poisoned OR poisoning OR poisonings OR sick OR sickness OR toxic OR toxicity OR diagnose OR diagnosis OR diagnosed OR diagnoses)

AND

(alpaca OR alpacas OR bronco OR broncos OR buffalo OR bull OR bulls OR cattle OR colt OR colts OR cow OR cows OR bovine OR bison OR oxen OR donkey OR donkeys OR duck OR ducks OR equine OR ewe OR ewes OR fillies OR filly OR foal OR foals OR gelding OR geldings OR heifer OR heifers OR horse OR horses OR lamb OR lambs OR livestock OR llama OR llamas OR mare OR mares OR mule OR mules OR mustang OR mustangs OR ponies OR pony OR ram OR rams OR sheep OR stallion OR stallions OR steer OR pig OR pigs OR piglet OR piglets)

Filters: English
Date search was conducted: 11/25/2015
Publication dates searched: 1/1/2012–11/25/2015

C.3 Search strategy focused on cats and birds

PubMed and Agricola Searches:

("*A. lemmermannii Raphidiopsis mediterranea*" OR *flos-aquae* OR anatoxin-a OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia* OR *Cyanobacteria* OR cyanotoxin OR Cyanotoxins OR "harmful algae" OR "harmful algal bloom" OR blue green algae)

AND

("health effect" OR "health endpoint" OR "health outcome" OR dead OR death OR deaths OR died OR disease OR diseased OR diseases OR exposed OR exposure OR ill OR illness OR illnesses OR infect OR infected OR infection OR infections OR morbidity OR mortality OR

poison OR poisoned OR poisoning OR poisonings OR sick OR sickness OR toxic OR toxicity OR diagnose OR diagnosis OR diagnosed OR diagnoses)

AND

(feline OR felines OR cat OR cats OR kitten OR kittens OR "*F. Catus*" OR "*Felis Catus*" OR bird OR birds OR avian OR waterfowl)

Filters: English
Date search was conducted: 2/1/2016
Publication dates searched: 1/1/2012–2/1/2016

Web of Science Search:

("*lemmermannii Raphidiopsis mediterranea*" OR *flos-aquae* OR anatoxin OR *Aphanizomenon* OR cylindrospermopsin OR "*C. raciborskii*" OR *Cuspidothrix* OR *Cylindrospermopsis* OR *Cylindrospermum* OR "*Cylindrospermopsis raciborskii*" OR *Dolichospermum* OR "*M. aeruginosa*" OR *Microcystis* OR microcystin OR microcystins OR *Oscillatoria* OR *Planktothrix* OR *Phormidium* OR *Tychonema* OR *Woronichinia* OR Cyanobacteria OR cyanotoxin OR Cyanotoxins OR "harmful algae" OR "harmful algal bloom" OR blue green algae)

AND

("health effect" OR "health endpoint" OR "health outcome" OR dead OR death OR deaths OR died OR disease OR diseased OR diseases OR exposed OR exposure OR ill OR illness OR illnesses OR infect OR infected OR infection OR infections OR morbidity OR mortality OR poison OR poisoned OR poisoning OR poisonings OR sick OR sickness OR toxic OR toxicity OR diagnose OR diagnosis OR diagnosed OR diagnoses)

AND

(feline OR felines OR cat OR cats OR kitten OR kittens OR "*F. Catus*" OR "*Felis Catus*" OR bird OR birds OR avian OR waterfowl)

Filters: English
Date search was conducted: 2/1/2016
Publication dates searched: 1/1/2012–2/1/2016

APPENDIX D. REVIEW OF THE STATE OF THE SCIENCE ON CYANOBACTERIAL CELLS HEALTH EFFECTS

D.1 Introduction

This appendix provides information gathered and reviewed to determine the state of the science on health effects from cyanobacterial cells. EPA conducted literature searches to identify studies relevant to the health effects from cyanobacterial cells. Detailed information on the design and implementation of these searchers is provided in Appendix C. Results from these literature searches were reviewed for relevance to cyanobacterial cell exposures and health effects. This appendix builds on the cyanobacterial bloom information included in the main document by discussing additional detail on the nature of cyanobacterial cells as stressors and, in particular, the health effects associated with exposures to cyanobacterial cells.

D.1.1 Animal Studies

Cyanobacterial cells cause allergenicity and irritation in animals, independent of whether the cyanobacterial cells produce toxin. Three animal studies (Shirai et al. 1986; Stewart et al. 2006c; Torokne et al. 2001) demonstrated hypersensitivity reactions and dermal and eye irritation in several species. Results from Torokne et al. (2001) indicated that hypersensitization reactions do not correlate with microcystin content. Although the number of studies is limited and different species were evaluated in each study, these studies provide evidence to support hypersensitivity reactions in animals from exposure to cyanobacteria when cyanotoxins are not present (Shirai et al. 1986; Torokne et al. 2001) and when they are (Stewart et al. 2006c).

Cyanobacteria bloom samples collected from five different lakes or ponds were tested for allergenic and irritative effects in guinea pigs and rabbits, respectively (Torokne et al. 2001). The microcystin content (presumed to be total LR, RR, and YR) ranged from not detected to 2.21 mg/g. To determine sensitization, guinea pigs were initiated with an intradermal injection of freeze-dried cyanobacteria followed 7 days later by topical application at the injection site. Sensitization was moderate to strong in 30–67 percent of guinea pigs and did not correlate with microcystin content. The *Aphanizomenon ovalisporum* sample (a nontoxin-producing strain) sensitized 91percent of the animals and was the strongest allergen. Skin irritation tests in albino rabbits showed slight or negligible irritation, except for *Aphanizomenon ovalisporum*, which showed moderate irritation. The eye irritation evaluation in rabbits was positive for four of the five samples containing *Microcystis*.

Shirai et al. (1986) reported that C3H/HeJ mice, immunized i.p. with either sonicated or live cells from a *Microcystis* water bloom, developed delayed-type hypersensitivity when challenged 2 weeks later with a subcutaneous injection sonicated *Microcystis* cells. A positive reaction, as assessed by footpad swelling, was seen in mice immunized with either live cells or sonicated cells. Both toxic and nontoxic *Microcystis* cells induced delayed-type hypersensitivity in this mouse study. Because this strain of mouse is unresponsive to lipopolysaccharide (LPS), the footpad delayed-type hypersensitivity was not related to LPS, thus, the antigenic component of the sonicated cyanobacterial cells is not known.

Stewart et al. (2006c) conducted a mouse ear swelling test in which cylindrospermopsin and *C. raciborskii* solutions generated irritation of the abdominal skin exposed during induction

(2 percent w/v lysed cell solution containing 73 μg/mL cylindrospermopsin). Subsequent dermal exposures to the *C. raciborskii* solution produced hypersensitivity reactions ($p = 0.001$). The cyanobacteria *Microcystis aeruginosa* and *Anabaena circinalis* elicited no responses in this test.

Two of the cyanobacterial cell studies in animals found that rodents became sensitized after exposure and subsequent challenge to nontoxin strains (Shirai et al. 1986; Torokne et al. 2001). Torokne et al. (2001) found that a nontoxic strain was more sensitizing and irritating than the toxic strains evaluated. These experiments support the conclusion that there is no relationship between the cyanotoxin content and the allergenic effect of cyanobacteria.

D.1.2 Clinical and Laboratory Human Studies

Several types of studies and reports provide information on associations between cyanobacteria exposure and health effects. Clinical and *in vitro* studies (Bernstein et al. 2011; Geh et al. 2015; Pilotto et al. 2004; Stewart et al. 2006a) have been able to assess associations between cyanobacteria exposure and human health effects including dermal and allergenic reactions. Three clinical studies assessed dermal exposure to cyanobacterial cells using skin-patch or skin-prick testing in humans (Bernstein et al. 2011; Pilotto et al. 2004; Stewart et al. 2006a). Some of the exposed individuals showed mild irritation or allergenicity. No statistically significant dose-response relationships were found between skin irritation and increasing cyanobacterial cell concentrations. The allergenicity study suggests that cyanobacteria are allergenic, particularly among people with chronic rhinitis (Bernstein et al. 2011).

Skin-patch testing in humans was performed by Pilotto et al. (2004) with laboratory-grown cylindrospermopsin-producing *C. raciborskii* cells, both whole and lysed, which were applied using adhesive patches at concentrations ranging from < 5,000 to 200,000 cells/mL to the skin of 50 adult volunteers. After 24 hours, patches were removed and evaluation of the erythematous reactions were graded. Analysis of participants' reactions to patches treated with whole cells showed an OR of 2.13 and a 95% Confidence Interval (CI) of 1.79–4.21 ($p < 0.001$). Lysed cells patch analysis showed an OR of 3.41 and a 95% CI of 2.00–5.84 ($p < 0.001$). No statistically significant increase or dose-response between skin reactions and increasing cell concentrations for either patches (whole or lysed) was observed. Subjects had skin reactions to the cylindrospermopsin, and positive control patches more frequently than to the negative control patches. The mean percentage of subjects with a reaction was 20% (95% CI: 15–31%). When subjects reacting to negative controls (39) were excluded, the mean percentage was 11percent (95% CI: 6–18%). Evaluation of erythematous reactions showed that mild irritations (grade 2) were resolved in all cases within 24 to 72 hours.

Stewart et al. (2006a) conducted a skin-patch test with 39 volunteers (20 dermatology outpatients; 19 controls) who were exposed to 6 cyanobacterial suspensions, including toxigenic species, nontoxigenic species, mixed suspensions, and two cyanobacterial LPS extracts. All cyanobacterial suspensions of lyophilized cells were tested at three concentrations, 0.25 percent w/v, 0.05 percent w/v, 0.005 percent w/v, and the estimated doses of cyanotoxins were 2.4 ng/kg cylindrospermopsin and 2.6 ng/kg microcystins. Only one subject showed significant responses to cyanobacterial suspensions, specifically to two suspensions of cyanobacterial cells: *C. raciborskii* and mixed *M. aeruginosa* and *C. raciborskii*, both of which contained one or more cyanotoxins. This subject showed no evidence of any dose-response effect in the dermal reactions. None of the participants reacted to the cyanobacterial LPS extracts, which ranged from

260 ppb to 31 ppm. This small clinical study demonstrated that dermal hypersensitivity reactions to cyanobacteria exposure occur infrequently, and further research into risk factors for predisposition to this type reaction could be beneficial.

Bernstein et al. (2011) studied skin sensitization to non-toxic extracts of *M. aeruginosa* in 259 patients with chronic rhinitis over 2 years. Patients were evaluated with aeroallergen skin testing and skin-prick testing. The authors found that 86 percent of the subjects had positive skin prick tests to *Microcystis aeruginosa*, and that patients with existing allergic rhinitis were more likely to have reactions and sensitization to cyanobacteria than the controls (non-atopic health subjects). This study indicated that cyanobacterial allergenicity is associated with the non-toxic portion of the cyanobacteria.

Geh et al. (2015) studied the immunogenicity of extracts of toxic and non-toxic strains of *M. aeruginosa* in patient sera (18 patients with chronic rhinitis and 3 non-atopic healthy subjects as documented in Bernstein et al. [2011]). Enzyme Linked Immunosorbent Assay (ELISA) test was used to test IgE-specific reactivity, and gel electrophoresis, followed by immunoblot and mass spectrometry, was done to identify the relevant sensitizing peptides. The authors found an increase in specific IgE in those patients tested with the non-toxic *Microcystis* extract than the extract from the toxic strain. After pre-incubation of the non-toxic extract with various concentrations of microcystin, the authors found that phycocyanin and the core-membrane linker peptide were responsible for the release of β-hexosaminidase in rat basophil leukemia cells. The authors concluded that non-toxin-producing strains of cyanobacteria are more allergenic than toxin-producing strains in allergic patients, and that the toxin may have an inhibitory effect on the allergenicity of the cyanobacterial cells.

D.1.3 Epidemiological Studies and Case Reports

Among the epidemiological studies discussed here, some identified significant associations between cyanobacteria exposure and a range of health outcomes including dermal, eye/ear, GI, and respiratory effects. Several of these studies also measured one or more cyanotoxins and found no association between cyanotoxin occurrence or exposure and health effects. Additional evidence from outbreak and case reports provides support for health effects associated with cyanobacteria exposure. Overall, these studies provide evidence of significant associations between cyanobacterial cell exposure and human health effects even in the absence of cyanotoxins. However, the reported associations between cyanobacterial cell densities and health outcomes are not consistent. The studies vary in study design, methods used, size of study population, cyanobacterial species evaluated, health effects identified, and cyanobacterial cell densities associated with human health effects. Therefore, substantial uncertainty remains regarding the associations between cyanobacterial cell exposure and human health effects.

Eight epidemiological studies evaluated short-term health effects associated with recreational exposure to cyanobacterial blooms (El Saadi et al. 1995; Lévesque et al. 2014; Lin et al. 2015; Philipp 1992; Philipp & Bates 1992; Philipp et al. 1992; Pilotto et al. 1997; Stewart et al. 2006d). See Table D-1 for a summary list of these studies. The health outcomes evaluated included dermal, GI, respiratory, and other acute effects, such as eye or ear symptoms. Seven studies evaluated recreational exposure to freshwater cyanobacteria, and one evaluated exposure to marine water cyanobacteria (Lin et al. 2015). Two studies included field sites in the continental United States or Canada (Lévesque et al. 2014; Stewart et al. 2006d), three occurred

in the United Kingdom (Philipp 1992; Philipp & Bates 1992; Philipp et al. 1992), and three were conducted in sub-tropical and tropical regions in Australia (El Saadi et al. 1995; Pilotto et al. 1997) and Puerto Rico (Lin et al. 2015). These epidemiological studies are discussed below in chronological order.

Table D-1. Cyanobacteria Epidemiological Studies Summary

Reference	Study Design, n, and Location	Cyanobacteria Identified	Cyanotoxins Measured	Health Association[a]	Lowest Significant Cyanobacterial Cell Density (cells/mL)
Philipp (1992)	Cross-sectional n = 246 UK (Hampshire)	*Microcystis* sp., *Gleotrichia* sp.	-	No statistically significant health associations	No quantitative cyanobacterial cell densities provided
Philipp and Bates (1992)	Cross-sectional n = 382 UK (Somerset)	*Microcystis* sp., *Gleotrichia* sp.	-	No statistically significant health associations	No quantitative cyanobacterial cell densities provided
Philipp et al. (1992)	Cross-sectional n = 246 UK (Lincolnshire, South Yorkshire)	*Oscillatoria* sp., *Aphanizomenon* sp., *Aphanothece* sp., *Merismopedia* sp.	-	No statistically significant health associations	No quantitative cyanobacterial cell densities provided
El Saadi et al. (1995)	Case-control n cases = 102 GI, 86 dermatological n controls = 132 Australia (South Australia)	*Anabaena* sp., *Aphanizomenon* sp., *Planktothrix* sp., *Anabaena circinalis*, *Microcystis aeruginosa*	-	No statistically significant health associations	No quantitative cyanobacterial cell densities provided
Pilotto et al. (1997)	Cross-sectional n = 295 exposed n = 43 unexposed Australia (South Australia, New South Wales, Victoria)	*Microcystis aeruginosa*, *Microcystis* sp., *Anabaena* sp., *Aphanizormenon* sp., *Nodularia spumigena*	Hepatotoxins detected by mouse bioassay	Significant positive association between combined symptoms (GI, dermal, respiratory, fever, eye or ear irritation) and cyanobacteria	> 5,000
Stewart et al. (2006d)	Cohort (prospective) n = 1,331 Australia (Queensland, New South Wales) and Florida	Cyanobacteria identified, species not specified	Microcystins detected by high-performance liquid chromatography (HPLC) with photodiode array detection or ELISA; cylindrospermopsin and anatoxin-a detected by HPLC-MS/MS; saxitoxins not detected by HPLC with fluorescence detection	Significant positive association between respiratory symptoms and cyanobacteria Significant positive association between combined symptoms (GI, dermal, respiratory, fever, eye or ear irritation) and cyanobacteria	> 100,000[b]
Lévesque et al. (2014)	Cohort (prospective) n = 466 Canada (Quebec)	Cyanobacteria identified, species not specified	Microcystins detected by ELISA	Significant positive association between GI symptoms with fever and cyanobacteria	20,000–100,000

Reference	Study Design, n, and Location	Cyanobacteria Identified	Cyanotoxins Measured	Health Association[a]	Lowest Significant Cyanobacterial Cell Density (cells/mL)
Lin et al. (2015)[c]	Cohort (prospective) n = 15,726 Puerto Rico (Boquerón)	Cyanophyte filament, *Pseudanabaena* sp., Picocyanophyte, *Synechococcus* sp., *Synechocystis* sp., Cyanophyte cell pair, *Phormidium* sp., *Lyngbya* sp., *Trichodesmium* sp., *Aphanothece* sp., *Johannesbaptistia* sp., *Komvophoron* sp., Cyanophyte colony, Cyanophyte unicell sphere	Lyngbyatoxin-a and debromo-aplysiatoxin measured but not detected by HPLC-MC	Significant positive association between respiratory illness and cyanobacteria other than picocyanobacteria	36.7–237.4
				significant positive association between rash and cyanobacteria other than picocyanobacteria	> 237.4

[a] Includes only significant associations between recreational cyanobacteria exposure and health effects.
[b] Values were converted from cyanobacterial cell surface area (>12.0 mm2/mL) to cyanobacterial cell density (>100,000 cells/mL) using conversions in NHMRC (2008). Relationship between biomass and cyanobacterial cell density can vary by species and cell size (Lawton et al. 1999; Stewart et al. 2006d).
[c] Lin et al. (2015) evaluated picocyanobacteria and cyanobacteria other than picocyanobacteria separately.

Three cross-sectional studies were conducted by Philipp et al. (Philipp 1992; Philipp & Bates 1992; Philipp et al. 1992) to evaluate health effects related to exposure to cyanobacteria from recreational activities including sailing, windsurfing, and fishing in water bodies in the United Kingdom. Questionnaires were administered to participants who visited one of six inland lakes to evaluate exposure and morbidity (including dermal, eye/ear, GI, and respiratory symptoms). Several species of cyanobacteria were identified and, in some cases, cyanobacterial levels exceeded the National Rivers Authority threshold for "potential to cause harm." Only minor morbidity was identified among recreators, and no statistically significant associations between cyanobacteria exposure and morbidity were identified.

El Saadi et al. (1995) conducted a case-control study in Australia to evaluate exposure to river water with detectable levels of cyanobacteria and GI and dermatological symptoms evaluated by a medical practitioner. This river was used as a source for drinking water, domestic water, and recreational water. The authors found no significant association between recreational exposure to river water with cyanobacteria and GI or dermatological symptoms. Cyanotoxins were not measured, but species of cyanobacteria were present that were capable of producing cyanotoxins.

These four earlier studies (El Saadi et al. 1995; Philipp 1992; Philipp & Bates 1992; Philipp et al. 1992) provided no quantitative data on cyanobacterial cell densities. Therefore, they could not help inform determination of a quantitative level associated (or not associated) with health effects.

Four more recent epidemiological studies assessed the association between exposure to recreational waters containing cyanobacteria and human health and provide quantitative density data for cyanobacterial cells (Lévesque et al. 2014; Lin et al. 2015; Pilotto et al. 1997; Stewart et al. 2006d). These studies reported at least one statistically significant association between

exposure to cyanobacteria and human health outcomes., including GI illness (Lévesque et al. 2014), respiratory symptoms (Lin et al. 2015; Stewart et al. 2006d), dermal symptoms (Lin et al. 2015), or combined symptomology (GI, dermal, respiratory, and other symptoms) (Pilotto et al. 1997; Stewart et al. 2006d). These associations were linked to a range of densities of cyanobacterial cells from as low as > 5,000 cells/mL (Pilotto et al. 1997) to as high as 100,000 cells/mL (analogous to ≥ 12 mm^2/mL (NHMRC 2008; Stewart et al. 2006d). In contrast to the studies that examined all cyanobacteria, Lin et al. (2015) evaluated picocyanobacteria, larger cyanobacterial cells, and total phytoplankton, and reported health effects associated with 37–1,461 cells/mL for cyanobacteria other than picocyanobacteria.

Pilotto et al. (1997) investigated the health effects from recreational exposures (including jet-skiing, water-skiing, swimming, and windsurfing) to cyanobacteria in Australia. The study included 852 participants, 777 who had water contact and were considered exposed, and 75 not exposed. There were 338 recreators (295 exposed, 43 not exposed) after exclusion of those who experienced symptoms or had recreational exposure in the 5 days prior to the initial interview at the water recreation site (the *after exclusion* study group). Health outcomes evaluated included diarrhea, vomiting, flu-like symptoms (e.g., cough), skin rashes, mouth ulcers, fevers, or eye or ear infections. Water samples were collected for evaluation of cyanobacterial cell counts, hepatotoxins, and neurotoxins.

In the *after exclusion* study group, when all symptoms were combined, the authors found a significant trend of increasing symptom occurrence with duration of exposure at 7 days post-exposure (*p-value* for trend =0.03). Similarly, in the *after exclusion* study group there was a significant trend of increasing symptom occurrence with increasing cyanobacterial cell count (*p-value* for trend = 0.04). To account for the combined effect of duration of exposure and cyanobacterial cell density, unexposed participants were compared with those exposed for up to 60 minutes and for more than 60 minutes to water with up to 5,000 cells/mL and to water with more than 5,000 cells/mL. For the *after exclusion* study group, a significant trend of increasing symptom occurrence with increasing levels of exposure was identified (*p-value* for trend =0.004). In addition, participants with recreational exposure for more than 60 minutes to cyanobacterial densities above 5,000 cells/mL had a significantly higher symptom occurrence rate at 7 days post-exposure than unexposed participants (OR = 3.44, CI: 1.09–10.82). In this study, the significant trends observed in the *after exclusion* study group were not observed when all participants were included.

Pilotto et al. (1997) reported toxicity data collected by the Australia Water Quality Center. Presence or absence of particulate (intracellular) hepatotoxins in concentrated surface water phytoplankton samples was measured by mouse bioassay. The authors reported that hepatotoxins were identified at one site on two separate interview days and at three sites for one day each. No evidence of neurotoxins was detected. They reported that no significant association was found between the presence of hepatotoxins and symptom occurrence at two and seven days after exposure. Data and analysis methods were not provided. The authors point out that trends were observed at seven days and not at two days after exposure and this might suggest a delayed rather than an immediate allergic response. The authors also stated they could not rule out other causative factors, such as other microorganisms, that could co-occur with cyanobacteria. The results from this study informed the recommendations made by WHO in *Guidelines for Safe Recreational Water Environments* (WHO 2003).

Stewart et al. (2006d) conducted a prospective cohort study to investigate the incidence of acute symptoms in individuals exposed to cyanobacteria via recreational activities in lakes and rivers in Australia and Florida. This study included 1311 recreators with any water contact-related activity (e.g., swimming, boat entry/egress). Cyanobacterial cell densities were characterized in terms of cell surface area rather than cell counts (to normalize for cell size differences among different species). Authors evaluated incidence of acute symptoms in recreators exposed to low, medium and high levels of cyanobacteria.

Study subjects were asked to complete a self-administered questionnaire before leaving for the day after enrollment and to submit to a telephone follow-up interview. The questionnaire and follow-up interview forms gathered information on various acute illnesses, their onset and severity. Respiratory symptoms among study participants in the high recreational exposure group (total cyanobacterial cell surface area > 12 mm^2/mL on day of recreation) were significantly greater compared to participants in the low recreational exposure group (< 2.4 mm^2/mL) (adjusted OR = 2.1, 95% CI: 1.1–4.0). Respiratory symptoms were defined as difficulty breathing, dry cough, productive cough, runny nose, unusual sneezing, sore throat, or wheezy breathing. Reports of any symptom among study participants in the high exposure group were significantly greater compared to reports among study participants in the low recreational exposure group (adjusted OR = 1.7, 95% CI: 1.0–2.9). However, when subjects with recent prior recreational water exposure were excluded the result remained positive but not significant (adjusted OR = 1.6, 95% CI: 0.8–3.2). A dose-response relationship between increased cyanobacterial biomass and increased symptom reporting was not identified. The authors speculated that the pattern in their data could be due to a threshold effect. No other significant associations with health effects were identified.

For water samples that contained potentially toxic cyanobacteria, Stewart et al. (2006d) measured cyanotoxins including microcystins, saxitoxins, cylindrospermopsin and anatoxin-a by HPLC or HPLC-MS/MS methods. Cyanotoxins were infrequently identified and only at low levels. Microcystins were detected on two occasions (1 and 12 µg /L). Cylindrospermopsin was found on seven occasions (ranging from 1-2 µg /L). Anatoxin-a was identified on a single recruitment day at a concentration of 1 µg/L. A statistically significant increase in symptom reporting was found to be associated with anatoxin-a exposure, but the number of exposed subjects was very low (n =18). No relationship between fecal indicator bacteria (fecal coliforms) and symptoms was identified.

Lévesque et al. (2014) conducted a prospective study of health effects including GI, respiratory, dermal, eye/ear, and other symptoms associated with cyanobacteria and microcystin exposure at three lakes in Canada (Quebec), one of which was a local supply of drinking water. The study evaluated acute symptoms in humans (466 subjects included in analysis) living in proximity to lakes affected by blooms and analyzed recreational exposure (full and limited contact) and drinking-water exposure scenarios for both cyanobacterial cells and microcystins.

More severe GI symptoms, defined as diarrhea, vomiting, nausea and fever, or abdominal cramps and fever, were associated with recreational contact (full and limited) and cyanobacteria. For the more severe GI symptoms, the adjusted relative risk RR increased with cyanobacterial cell counts providing evidence of a dose-response relationship (*p-value* for trend= 0.001, < 20,000 cells/mL: RR = 1.52, 95% CI: 0.65–3.51; 20,000–100,000 cells/mL: RR = 2.71, 95% CI: 1.02–7.16; > 100,000 cells/mL: RR = 3.28, 95% CI: 1.69–6.37). No evidence of a dose-response relationship for cyanobacterial cell counts and the less severe GI symptoms was found.

No relationship was observed between duration of contact or head immersion and risk of GI symptoms. A significant increase for both the less and the more severe GI symptoms was found with contact in the more highly impacted lakes (median cell densities 20,001–21,485 cells/mL), but not in the less impacted lake (median 1,032 cells/mL). No relationship was observed between microcystin concentrations and risk of GI symptoms. No significant associations between recreational exposures to cyanobacteria and health effects other than GI effects were identified.

To evaluate possible co-exposures, authors measured microcystin concentrations and *E. coli* as a fecal indicator. Lévesque et al. (2014) measured particulate (intracellular) and dissolved microcystins by ELISA and found that microcystins concentrations varied by lake and by sample location (littoral vs. limnetic). Microcystin was detected in all three lakes. At Lake William the median values were below the limit of detection at littoral and limnetic stations, with maximum values of 0.63 μg/L and 0.02 μg/L respectively. At Lake Roxton littoral stations, the median concentration was 0.23 μg/L (range: 0.008 μg/L–108.8 μg/L) and at limnetic stations the median was 0.12 μg/L (range: 0.04 μg/L–1.12 μg/L). The Mallets Bay littoral stations had a median of 0.70 μg/L (range: under limit of detection – 773 μg/L) and the limnetic stations had a median of 0.35 μg/L (range: 0.001 μg/L–125 μg/L).

Lévesque et al. (2014) reported that as a whole the microcystin concentrations during contact were relatively low (1st tertile: < 0.0012 μg/L; 2nd tertile: 0.0012–0.2456 μg/L; 3rd tertile: > 0.2456 μg/L). Symptoms were examined in relation to recreational and drinking water exposure to cyanobacteria and microcystin. Only GI symptoms were associated with recreational contact. The highest concentration of microcystin at which an episode of GI symptoms was reported was 7.65 μg/L. There was no significant increase in adjusted relative risk of GI symptoms with recreational exposure to more than 1 μg/L microcystin. Adjusted relative risks (adjusted for gender, gastrointestinal symptoms reported in the two weeks prior to data collection, residence's source of drinking water) for GI illness without fever and GI illness with fever were 1.06 (95% CI=0.32–3.52) and 1.48 (95% CI = 0.41–5.23), respectively. There were significant increases in adjusted relative risk of several symptoms in participants who received their drinking water from a source contaminated by cyanobacteria (muscle pain, GI illness, skin, and ear symptoms).

Lévesque et al. (2014) found that the geometric mean of *E. coli* at the three lakes ranged from 0 to 145 CFU per 100 mL, and there was no association between GI illness and *E.coli* levels. The authors noted that GI symptoms could have other causes, such as *Aeromonas* infections; however, the symptoms were not related to fecal contamination as measured by culturable *E. coli*. They also noted that people avoided full recreational contact during blooms and more people engaged in limited contact recreation at higher cell counts. This observation explains the counterintuitive finding that participants with limited contact exposure (fishing, watercraft without direct water contact) had higher likelihood of symptom reporting compared to participants with full contact.

A follow-up analysis (Lévesque et al. 2016) characterized the same health data as Lévesque et al. (2014) to evaluate the relationship of bacterial endotoxin (lipopolysaccharides or LPS) concentration to GI symptoms. Endotoxin concentrations were slightly correlated with cyanobacterial counts (polychoric correlation coefficient = 0.57). The highest tertile of endotoxin concentration (> 48 endotoxin units/mL) was significantly associated with GI illness both with and without fever (GI illness without fever relative risk (RR) = 2.87, CI: 1.62–5.08; GI illness with fever RR = 3.11, CI: 1.56–6.22). Adjustment to the level of cyanobacteria did not

alter the relationship between endotoxin and GI illness and authors hypothesize that other gram negative bacteria might play a role in the relationship between endotoxin levels and GI illness as has been suggested in a previous study (Berg et al. 2011). Authors note that they stored filtered water samples at -80 ºC for several months prior to conducting endotoxin testing and that another study (O'Toole et al. 2009) showed a 44 percent mean decline in the concentration of endotoxins in samples stored at -80 ºC for several weeks compared to samples stored at 4 ºC for 24 hours. Lévesque et al. (2016) caution that concentrations reported could be underestimated and should be interpreted on an ordinal basis. Two other studies conducting endotoxin testing on frozen samples found concentrations of a similar magnitude as this study (Berg et al. 2011; Rapala et al. (2002).

Lin et al. (2015) conducted a prospective study based on data collected in 2009 at Boquerón, Puerto Rico for 26 study days involving 15,726 enrollees to examine the association between phytoplankton cell counts and illness among beachgoers. Three categories of phytoplankton were evaluated: picocyanobacteria, cyanobacteria other than picocyanobacteria, and total phytoplankton. The analysis compared people exposed at phytoplankton cell count levels > 25th percentile (e.g., 25th to 75th percentile, > 75th percentile) to people exposed at levels < 25th percentile (range of cyanobacteria other than picocyanobacteria: < 37–1461 cells/mL).

The study reported significant associations between recreational exposure to cyanobacteria other than picocyanobacteria and respiratory symptoms, rash, and earache. For the other symptoms measured, including eye irritation, no significant associations were observed. More specifically, cyanobacterial (other than picocyanobacterial) densities of 37 to 237 cells/mL (> 25th to < 75th percentile) and densities ≥ 237 cells/mL (≥ 75th percentile) were associated with increased respiratory symptoms (> 25th to < 75th percentile, odds ratio (OR) = 1.30, 95% CI = 1.08–1.56; ≥ 75th percentile, OR = 1.37, 95% CI = 1.12–1.67) in study participants who reported body immersion. Respiratory symptom occurrence was defined as any two of the following: sore throat, cough, runny nose, cold, or fever. Cyanobacterial (other than picocyanobacterial) densities >237 cells/mL were associated with rash (OR = 1.32, 95% CI = 1.05–1.66) and earache (OR = 1.75, 95% CI = 1.09–2.82). Study participants who reported head submersion or swallowing of water showed no relationship between recreational exposures to cyanobacteria (other than picocyanobacteria) and respiratory symptoms. There was no association between recreational exposures to cyanobacteria (other than picocyanobacteria) and respiratory symptoms in study participants who reported head submersion or swallowing of water. A statistically significant association between cyanobacterial cell exposure (other than picocyanobacterial cell exposure) and all health effects combined was also observed.

Lin et al. (2015) measured the dermatotoxins, debromoaplysiatoxin and lyngbyatoxin, using high performance liquid chromatography-mass spectrometry and did not detect levels above the limit of detection of 1.0 ppb. Authors reported that debromoaplysiatoxin and lyngbyatoxin-a are photolabile and are unlikely to persist in the water column (Moikeha & Chu 1971). They noted that the health effects identified in this study were consistent with previous blooms of *Lyngbya majuscula*, which can produce these toxins, though *Lyngbya* only comprised 3 percent of total planktonic cyanobacteria (other than picocyanobacteria). It is also possible that the cyanobacterial cells could be having direct health effects as cyanotoxins levels were below the limit of detection.

To evaluate possible co-exposures, some studies measured cyanotoxins and fecal indicators. Lin et al. (2015), Lévesque et al. (2014), Pilotto et al. (1997), and Stewart et al. (2006d) measured one or more cyanotoxins or total hepatotoxins. In some cases, cyanotoxin levels were below the limit of detection. To determine if study participants possibly were exposed to fecal contamination, three of the studies (Lévesque et al. 2014; Lin et al. 2015; Stewart et al. 2006d) measured bacterial fecal indicators at some study locations and times. Of the studies that measured bacterial fecal indicators, none found an association between bacterial fecal indicators and health effects. Of these studies, the only one with data available for viral fecal indicators or concentrations of waterborne pathogens was Lin et al. (2015) provided in Wade et al. (2010) and Soller et al. (2016).

In summary, although four studies identified significant associations between cyanobacteria exposure and health effects, the type of health effect identified varied. One study reported a significant association between GI illness and exposure to cyanobacteria (Lévesque et al. 2014). Stewart et al. (2006d) and Lin et al. (2015) identified statistically significant associations between cyanobacterial cell exposure and respiratory effects. Lin et al. (2015) also found a statistically significant association between earache and cyanobacterial densities (other than picocyanobacteria). Both Pilotto et al. (1997) and Stewart et al. (2006d) found statistically significant associations between cyanobacterial cell exposure and all symptoms combined. The three cross-sectional studies conducted in the United Kingdom in 1990 found no statistically significant associations, although some minor elevated morbidity was observed in exposed individuals (Philipp 1992; Philipp & Bates 1992; Philipp et al. 1992). Another 1992 case-control epidemiological study in Australia found no statistically significant symptoms for exposed recreators (El Saadi et al. 1995).

The Centers for Disease Control and Prevention (CDC) has collected information on illness outbreaks associated with HABs, which commonly involve cyanobacteria. This information includes human health effects and water-sampling results voluntarily reported to the Waterborne Disease Outbreak Surveillance System via the National Outbreak Reporting System and the Harmful Algal Bloom Related Illness Surveillance System. CDC published summary information on HAB-associated outbreaks from recreational exposures focusing on 2009–2010 with limited additional information available for outbreaks that occurred in 2001, 2004, and 2011–2012 (Dziuban et al. 2006; Hilborn et al. 2014; Hlavsa et al. 2014; Yoder et al. 2004). CDC defines a recreational water-associated outbreak as the occurrence of similar illnesses in two or more persons, epidemiologically linked by location and time of exposure to recreational water or recreational water-associated chemicals volatilized into the air surrounding the water.

The 2009–2010 reporting cycle was notable, as almost half (46 percent) the recreational water outbreaks reported to CDC were associated with HABs (Hilborn et al. 2014). Three of the outbreaks confirmed the presence of cyanobacteria, and four confirmed the presence of cyanotoxins. Gastrointestinal and dermatologic symptoms were the most commonly reported symptom categories associated with HAB-related outbreaks in freshwater (Dziuban et al. 2006; Hilborn et al. 2014; Hlavsa et al. 2014; Yoder et al. 2004). For the cyanobacteria-associated outbreaks with reported symptom counts, the most common symptoms reported were GI related, including vomiting, diarrhea, and nausea (estimated to be > 40 percent). The second most frequent outbreak symptom reported was skin rash (> 27 percent cases reported). Fever, earache, skin irritation, and headache were the next most frequently reported symptoms (11 percent, 9 percent, and 9 percent of cases reported, respectively).

Hilborn et al. (2014) analyzed the HAB outbreak data from 2009–2010 and found 66 percent of case patients were individuals aged 1–19 years (n = 38 of 58 total) and 35 percent were aged 9 years or younger (n = 20). In addition, in a cyanobacteria-associated outbreak in 2001, 42 children were affected. These data are limited and might be underreported, but they suggest that children could be at increased risk for cyanobacteria-associated illness via recreational exposure.

In addition to reports related to freshwater exposure, health effects including dermal, eye/ear, and respiratory effects have been reported following exposure to marine cyanobacteria and/or cyanotoxins including *Lyngbya majuscula* which can produce the cyanotoxins lyngbyatoxin A and debromoaplysiatoxin (Osborne & Shaw 2008).

D.2 Mode of Action

Few mechanistic investigations have been completed on how exposure to cyanobacterial cells might lead to inflammatory response. Torokne et al. (2001) evaluated the sensitization and irritation potential of *Microcystis*, *Anabaena*, *Cylindrospermopsis*, and *Aphanizomenon* bloom and strain samples and found no correlation between the cyanotoxin content and allergenicity. For example, the nontoxic *Aphanizomenon* was the most allergenic sample, more allergenic than the most toxic cyanobacterial cells they studied, *Microcystis aeruginosa*. Stewart et al. (2006e) concluded that cutaneous effects strongly suggest allergic reactions, and symptoms such as rhinitis, conjunctivitis, asthma, and urticaria (or hives) also indicate immediate hypersensitivity responses, which are probably explained by a cascade action of pro-inflammatory cytokines.

Bernstein et al. (2011) suggested that the allergenic structure of cyanobacteria might be associated with a nontoxin-producing part of the organism. Building on this conclusion, Geh et al. (2015) conducted a series of experiments to identify the cyanobacteria allergen(s) responsible for sensitization. Study participants were given skin-prick tests with extracts from nontoxic *M. aeruginosa* strains. Serum from these individuals was collected from a subset of 15 patients who elicited strong skin test responses to *M. aeruginosa* and from 3 healthy control subjects. The lysate from nontoxic *M. aeruginosa* strains was significantly ($p < 0.01$) more immunoreactive than the lysate from the toxin-producing strains, which suggests that the nontoxic strain was more allergenic than the toxic strain. They found, however, that IgE binds to *M. aeruginosa* peptides present in lysates of both the toxic and nontoxic strains. Geh et al. (2015) also performed a β-hexosaminidase release assay, as a surrogate assay for measuring histamine release, to identify functional activity of the *M. aeruginosa* extracts using rat basophil leukemia cells. The authors concluded that the same allergen is present in toxic and nontoxic *M. aeruginosa* lysates, but suggest the toxic *M. aeruginosa* lysate might contain an endogenous inhibitor that prevents IgE from effectively binding to the specific allergen. The further analysis by Geh et al. (2015) of the sera of individuals exposed to nontoxic *M. aeruginosa* lysate indicated that either linker core-membrane peptide or phycocyanin, or both, are potentially responsible for *M. aeruginosa* allergenicity.

Epidemiological studies and case reports suggest respiratory effects that could be consistent with an allergic or hay fever type reaction (Giannuzzi et al. 2011; Stewart et al. 2006e). Inhalation exposure to bacterial endotoxins (i.e., a toxin that is part of the cyanobacterial cell as opposed to exotoxins such as microcystins and cylindrospermopsin) has been found to be associated with pulmonary disease, including asthma, chronic obstructive airway disease, and

emphysema (Stewart et al. 2006b). A recent review of the structure and effects of cyanobacterial lipopolysaccharide suggested that it could act as an antagonist of the TLR4 receptor and inhibit the inflammatory response pathway (Durai et al. 2015).

Stewart et al. (2006e) also noted that, although symptoms and time to onset can be disparate, several reports described:

> "a collective group of symptoms resembling immediate or Type-I hypersensitivity reactions. Immediate hypersensitivity reactions are commonly associated with atopy, which is the familial tendency to react to naturally occurring antigens, mostly proteins, through an IgE-mediated process. Atopy frequently manifests as a spectrum of diseases, e.g., seasonal rhinitis, conjunctivitis, asthma, and urticaria."

Documentation of this type of respiratory response is consistent with results from Geh et al. (2015) and further supports that immune system response follows exposure to cyanobacteria.

In older literature, cyanobacterial lipopolysaccharide was suspected as being a cause of inflammatory response because this cell structure, also found in many gram-negative bacterial species, has been observed to initiate acute inflammatory responses in mammals that are typical of a host reaction to tissue injury or infection (Stewart et al. 2006b). The Stewart et al. (2006e) review, however, found evidence to support this mechanism lacking. Although all cyanobacteria contain the pigment phycocyanin, not all species of cyanobacteria have shown dermal reactions. Also, some species of cyanobacteria produce toxins that are known dermal irritants (e.g., lyngbyatoxin-a). Pilotto et al. (2004), however, found that 20–24 percent of the study participants exposed to cyanobacterial cells via skin patches for 24 hours showed dermal reactions to cyanobacteria species, both whole and lysed cells.

Stewart et al. (2006b) noted that the effects of microcystin- and cylindrospermopsin-producing bacteria on the GI tract could suggest that cyanotoxins and lipopolysaccharide from the cyanobacteria or other bacteria residing in the gut might cross a gut mucosal barrier that has been disrupted and enhance the adverse effects of cyanotoxins.

An aquatic invertebrate study using brine shrimp (*Artemia salina, Daphnia magna* and *Daphnia galeata*) to determine the toxicity of microcystin and cylindrospermopsin in combination with cyanobacterial lipopolysaccharide found that pre-exposure to LPS increased the lethal concentration (LC_{50}) of cylindrospermopsin 8-fold (Lindsay et al. 2006). The authors concluded that the decrease in susceptibility to cylindrospermopsin was due to the effects of lipopolysaccharide on detoxification enzyme pathways; lipopolysaccharide decreased toxic metabolites of cylindrospermopsin by suppressing the invertebrate cytochrome P450 system, thus decreasing toxicity.

D.3 References

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APPENDIX E. INCIDENTAL INGESTION EXPOSURE FACTOR COMBINED DISTRIBUTION ANALYSIS

EPA combined the distributions of incidental ingestion rate per hour and recreational exposure duration to generate a hybrid distributions using R version 3.3.1. Table E-1 presents the parameters used to fit the distributions, and Table E-2 provides summary statistics for the combined distributions. The R code follows these tables.

Table E-1. Parameters Used to Fit Distributions

Parameter	Ingestion rate (L/hr)	Exposure duration (hr/d)
Mean	0.0501	2.737
Standard deviation	0.0401	1.733
Minimum		0.417
Maximum		7.5
μ (ln transformed)	-3.241	
σ^2 (ln transformed)	0.704	
Minimum (ln transformed)	-10^{10}	
Maximum (ln transformed)	-1.59	

Table E-2. Summary Statistics of Hybrid Distribution

Combined Distribution #	Ingestion rate (L/hr)*	Exposure Duration (hr/d)*	Summary Statistics for Ingestion (L/d)							
			Min	Q1	Median	Mean	Q3	Max	Percentile at 0.33 L/d	Percentile at 0.60 L/d
1	Normal	Normal	0.0000	0.0702	0.1424	0.1768	0.2465	1.2850	0.86	0.99
2	LN	Normal	0.0023	0.0564	0.1051	0.1448	0.1872	1.5340	0.91	0.99
3	LN	LN	0.0023	0.0479	0.0873	0.1241	0.1580	1.4730	0.94	0.99
4	LN	Gamma	0.0018	0.0467	0.0888	0.1281	0.1646	1.4580	0.93	0.99
5	Gamma	Gamma	0.0000	0.0396	0.0871	0.1307	0.1736	1.4020	0.92	0.99

* All input distributions were truncated to reflect observed minimum and maximum values

R Code

```
#Cyanotoxin recAWQC WA

#This script is to combine two distributions and generate histogram using five different distribution combinations

rm(list=ls()); # Remove all current R objects from memory

library(truncnorm) #import library for truncated normal distribution
```

```
# Convert exposure data from min/day to hr/day
mean_dur_min<-164.2 #mean exposure duration min/day
sd_dur_min<-103.97 #sd exposure duration min/day
med_dur_min<-140 #median exposure duration min/day
min_dur_min<-25 #minimum exposure duratrion min/day
max_dur_min<-450 #maximum exposure duration min/day

mean_dur<-mean_dur_min/60 #mean exposure duration hr/day
sd_dur<-sd_dur_min/60 #sd exposure duration hr/day
med_dur<-med_dur_min/60 #median exposure duration hr/day
min_dur<-min_dur_min/60 #minimum exposure duration hr/day
max_dur<-max_dur_min/60 #maximum exposure duration hr/day

#(1) Truncated normal ingestion and normal exposure duration distribution
mean_ing <- 0.05 #mean ingestion rate L/hr
sd_ing <- 0.04 #sd ingestion rate L/hr
min_ing<- 0 #minimum ingestion rate L/hr
max_ing<-0.205 #maximum ingestion rate L/hr

n = 100000 #number of samples

ingperhr_trunc<-rtruncnorm(n, a=min_ing, b=max_ing, mean_ing, sd_ing)

duration_hr_trunc<-rtruncnorm(n, a=min_dur, b=max_dur, mean_dur, sd_dur)

ingperday_trunc<-ingperhr_trunc*duration_hr_trunc

summary(ingperday_trunc)

hist(ingperday_trunc,xlab="Ingestion rate (L/day)",ylab="Frequency", main ="Normal distribution fit, truncated", xlim=c(0, 1.0), ylim=c(0, 400))
h=hist(ingperday_trunc)
h$density=h$counts/sum(h$counts)
plot(h,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated Normal distribution fit", xlim=c(0, 1), ylim=c(0, 0.6), xaxp=c(0,1.5,15), freq=FALSE)

#Determine percentiles in combined normal distribution
```

```
#(a)  97th ingestion rate =0.12 L/hr and mean exposure duration of 2.74 hr/day = 0.33 L/day

ecdf(ingperday_trunc)(0.33)

#(b)  97th ingestion rate =0.12 L/hr and 90th exposure duration of 5.0 hr/day = 0.60 L/day

ecdf(ingperday_trunc)(0.60)

#(2) Truncated Log-normal ingestion and normal exposure duration distribution

#transform mean and std of ingestion rate

sd_ing_ln<-sqrt(log((sd_ing/mean_ing)^2+1))

mean_ing_ln<-log(mean_ing)-((sd_ing_ln^2)/2)

min_ing_ln<- -10^10

max_ing_ln<-log(max_ing)

ingperhr_ln_trunc<-exp(rtruncnorm(n, a=min_ing_ln, b=max_ing_ln, mean=mean_ing_ln, sd=sd_ing_ln)) #truncated log normal distribution

duration_hr_trunc<-rtruncnorm(n, a=min_dur, b=max_dur, mean=mean_dur, sd=sd_dur) #truncated normal distribution

ingperday_ln_trunc<-ingperhr_ln_trunc*duration_hr_trunc #combine distributions

summary(ingperday_ln_trunc) #summary statistics about the combined distribution

#Generate histogram

hist(ingperday_ln_trunc,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated hybrid distribution fit", xlim=c(0, 2.0), ylim=c(0, 1))

h=hist(ingperday_ln_trunc)

h$density=h$counts/sum(h$counts)

plot(h,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated LN-Normal hybrid distribution fit", xlim=c(0, 1), ylim=c(0, 0.6), xaxp=c(0,1.5,15), freq=FALSE)

#Generate empirical cumulative distribution function

plot(ecdf(ingperday_ln_trunc), main="")

#Determine percentiles in combined distribution

#(a)  97th ingestion rate =0.12 L/hr and mean exposure duration of 2.74 hr/day = 0.33 L/day

ecdf(ingperday_ln_trunc)(0.33)

#(b)  97th ingestion rate =0.12 L/hr and 90th exposure duration of 5.0 hr/day = 0.60 L/day
```

```
ecdf(ingperday_ln_trunc)(0.60)

#(3) Truncated Log-normal ingestion and log-normal duration

sd_dur_ln<-sqrt(log((sd_dur/mean_dur)^2+1))

mean_dur_ln<-log(mean_dur)-((sd_dur_ln^2)/2)

min_dur_ln<-log(min_dur)

max_dur_ln<-log(max_dur)

ingperhr_ln_trunc<-exp(rtruncnorm(n=n, a=min_ing_ln, b=max_ing_ln,
mean=mean_ing_ln, sd=sd_ing_ln)) #truncated log normal distribution

duration_hr_ln_trunc<-exp(rtruncnorm(n=n, a=min_dur_ln, b=max_dur_ln,
mean=mean_dur_ln, sd=sd_dur_ln))

ingperday_ln2_trunc<-ingperhr_ln_trunc*duration_hr_ln_trunc #combine distributions

summary(ingperday_ln2_trunc) #summary statistics about the combined distribution

#Generate histogram

hist(ingperday_ln2_trunc,xlab="Ingestion rate (L/day)",ylab="Probability", main
="Truncated hybrid distribution fit", xlim=c(0, 2.0), ylim=c(0, 1))

h=hist(ingperday_ln2_trunc)

h$density=h$counts/sum(h$counts)

plot(h,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated log-normal
distribution fit", xlim=c(0, 1), ylim=c(0, 0.6), xaxp=c(0,1.5,15), freq=FALSE)

#Generate emperical cumulative distribution function

plot(ecdf(ingperday_ln2_trunc), main="")

#Determine percentiles in combined distribution

#(a)    97th ingestion rate =0.12 L/hr and mean exposure duration of 2.74 hr/day = 0.33
L/day

ecdf(ingperday_ln2_trunc)(0.33)

#(b)    97th ingestion rate =0.12 L/hr and 90th exposure duration of 5.0 hr/day = 0.60
L/day

ecdf(ingperday_ln2_trunc)(0.60)


# (4) Truncated log-normal ingestion distribution and gamma duration distribution (beta
distribution for duration added by arun)

vr_dur<- sd_dur^2 # variance of the duration distribution

theta_dur<-vr_dur/mean_dur # scale parameter of the gamma distribution

k_dur<-mean_dur/theta_dur # shape parameter of the gamma distribution

rgamma_trunc<-function(n,k,theta,min,max){
```

```
 i<-1
 gv<-matrix(,n,1)
 while(i<=n) {
 a<-rgamma(1,shape=k,scale=theta)
 if (a>min & a<max){
  gv[i]<-a
  i<-i+1 } # end of if operation
 } # end of while loop
 return(as.vector(gv))
 } # end of function
duration_hr_gm<-rgamma_trunc(n,k_dur,theta_dur,min_dur,max_dur)  #truncated gamma distribution
ingperday_ln_gm<-ingperhr_ln_trunc*duration_hr_gm #combine ln and gm distributions
summary(ingperday_ln_gm) #summary statistics about the combined distribution
#Generate histogram
hist(ingperday_ln_gm,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated hybrid distribution fit", xlim=c(0, 2.0), ylim=c(0, 1))
h=hist(ingperday_ln_gm)
h$density=h$counts/sum(h$counts)
plot(h,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated LN-Gamma hybrid distribution fit", xlim=c(0, 1), ylim=c(0, 0.6), xaxp=c(0,1.5,15), freq=FALSE)
#Generate emperical cumulative distribution function
plot(ecdf(ingperday_ln_gm), main="")
#Determine percentiles in combined distribution
#(a)     97th ingestion rate =0.12 L/hr and mean exposure duration of 2.74 hr/day = 0.33 L/day
ecdf(ingperday_ln_gm)(0.33)

#(b)     97th ingestion rate =0.12 L/hr and 90th exposure duration of 5.0 hr/day = 0.60 L/day
ecdf(ingperday_ln_gm)(0.60)
# (5) Truncated gamma ingestion distribution and gamma duration distribution
vr_ing<- sd_ing^2 # variance of the duration distribution
theta_ing<-vr_ing/mean_ing # scale parameter of the gamma distribution
k_ing<-mean_ing/theta_ing # shape parameter of the gamma distribution
```

```
duration_hr_gm<-rgamma_trunc(n,k_dur,theta_dur,min_dur,max_dur)  #truncated gamma distribution

ingperhr_gm<-rgamma_trunc(n,k_ing,theta_ing,min_ing,max_ing)  #truncated gamma distribution

ingperday_gm<-ingperhr_gm*duration_hr_gm #combine ln and gm distributions

summary(ingperday_gm) #summary statistics about the combined distribution

#Generate histogram

hist(ingperday_gm,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated hybrid distribution fit", xlim=c(0, 2.0), ylim=c(0, 1))

h=hist(ingperday_gm)

h$density=h$counts/sum(h$counts)

plot(h,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated Gamma distribution fit", xlim=c(0, 1), ylim=c(0, 0.6), xaxp=c(0,1.5,15), freq=FALSE)

#Generate emperical cumulative distribution function

plot(ecdf(ingperday_gm), main="")

#Determine percentiles in combined distribution

#(a)     97th ingestion rate =0.12 L/hr and mean exposure duration of 2.74 hr/day = 0.33 L/day

ecdf(ingperday_gm)(0.33)

#(b)     97th ingestion rate =0.12 L/hr and 90th exposure duration of 5.0 hr/day = 0.60 L/day

ecdf(ingperday_gm)(0.60)
```

APPENDIX F. INFORMATION ON CELLULAR CYANOTOXIN AMOUNTS AND CONVERSION FACTORS

The information in the tables below was generated from a brief survey of the peer-reviewed and published scientific literature. This survey was not a formal systematic literature search and was conducted to evaluate the availability of data needed to calculate a cyanobacterial cell density potentially associated with a specific cyanotoxin concentration.

Table F-1. Cell Quotas for Cyanotoxins Available from a Spot Check of the Literature

Toxin	Species	Site/Clone	Toxin Quota	Reference	Notes
Cylindrospermopsin	*Cylindrospermopsis raciborskii*	16 sites in 3 reservoirs in Queensland, Australia	4.5 – 55.8 fg[a] $cell^{-1}$; 10.0 – 49.4 fg $cell^{-1}$	Orr et al. (2010)	has values for cylindrospermopsin and d-cylindrospermopsin as cell versus cylindrospermopsin concentration in table
	Cylindrospermopsis raciborskii	New South Wales, Australia	31 (12 – 52) fg $cell^{-1}$	Hawkins et al. (2001)	also has biomass conversions (Table 2)
	Cylindrospermopsis raciborskii	Saudi Arabia lake	0.6 – 14.6 pg[b] $cell^{-1}$	Mohamed and Al-Shehri (2013)	
	Cylindrospermopsis raciborskii	Queensland, Australia	13.4 (± 2.6) – 14.9 (± 3.4) fg $cell^{-1}$	Davis et al. (2014)	range is for two strains
	Cylindrospermopsis raciborskii		12.1 (5.6) – 24.7 (9.5) ng[c] 10^{-6} cells; 3.2 (0.67) – 5.7 (1.4) ng 10^{-6} cells; 0.049 (0.002) – 0.094 (0.001) cylindrospermopsin chlorophyll a^{-1}; 0.016 (0.005) – 0.11 (0.003) cylindrospermopsin chlorophyll a^{-1}	Carneiro et al. (2013)	
	Cylindrospermopsis raciborskii	Queensland, Australia	0.28 x 10^{-2} (0.2 x 10^{-2}) – 1.8 x 10^{-2} (0.4 x 10^{-2}) pg $cell^{-1}$	Willis et al. (2015)	
	Cylindrospermopsis raciborskii	Queensland, Australia	19 (3) – 26 (4) fg $cell^{-1}$; 416 (67) – 447 (69) 10^3 fg μm^{-3}	Pierangelini et al. (2015)	breaks out cylindrospermopsin and d-cylindrospermopsin
Microcystin (MC)	*Planktothrix agardhii*	Paris, France lake	1.5 – 19 fg MC-LR $cell^{-1}$	Briand et al. (2008)	
	Planktothrix agardhii	England; Turkey	0.7 – 1.9 fg μm^{-3}; 75.6 – 91.2 fg $cell^{-1}$	Akcaalan et al. (2006)	
	Planktothrix rubescens	England; Turkey	1.4 – 2.9 fg μm^{-3}; 103.9 – 235.6 fg $cell^{-1}$	Akcaalan et al. (2006)	
	Planktothrix rubescens	Italy lake	1.0 – 3.9 μg mm^{-3};	Salmaso et al. (2014)	
	Planktothrix rubescens	France lake	0.13 (0.16) – 0.16 (0.27) pg $cell^{-1}$	Briand et al. (2008)	

Toxin	Species	Site/Clone	Toxin Quota	Reference	Notes
Microcystin (continued)	Model Cyanobacteria		91.5 fg cell^{-1}	Jähnichen et al. (2001)	Cites Long et al. (2001), Orr and Jones (1998), Jähnichen et al. (2001), and Watanabe et al. (1989) for quotas
	Microcystis aeruginosa	MASH01-A19	50 – 170 fg cell^{-1}	Orr and Jones (1998)	estimated from Figure 5
	Microcystis aeruginosa	Lake Huron, United States	140 fg cell^{-1}	Fahnenstiel et al. (2008)	
	Microcystis aeruginosa	Portugal lake	0.06 – 0.22 pg cell^{-1}	Vasconcelos et al. (2011)	
	Microcystis aeruginosa		18 (0.95) – 23.7 (0.96) fg cell^{-1}	Jähnichen et al. (2007)	
	Microcystis aeruginosa	PCC 7806	34.5 – 81.4 fg cell^{-1}	Wiedner et al. (2003)	
	Microcystis aeruginosa	France	0.05 – 3.8 pg cell^{-1}	Sabart et al. (2010)	estimated from Figure 3
	Microcystis aeruginosa	New Zealand	0.1 – 1.55 pg cell^{-1}	Wood et al. (2012)	estimated from Figure 1

[a] fg = femtogram
[b] pg = picogram
[c] ng = nanogram

Table F-2. A Brief Summary of Cell Concentration – Cyanotoxin Conversions Available from a Spot Check of the Literature

Toxin	Species	Site/Clone	Conversion	Reference	Notes
Cylindrospermopsin	*Cylindrospermopsis raciborskii*	16 sites in 3 reservoirs in Queensland, Australia	has cell versus cylindrospermopsin concentration in table	Orr et al. (2010)	additional data available but need to be digitized
	Cylindrospermopsis raciborskii	New South Wales, Australia	0.13% (0.06 – 0.35%) dry weight; 0.57 (0.18 – 1.52 %) fg μm^3 biovolume	Hawkins et al. (2001)	
	Cylindrospermopsis raciborskii	Queensland, Australia	1% w/w (10 mg cylindrospermopsin per g of dry weight) to 1 µg/g of dry weight	Eaglesham et al. (1999)	has cylindrospermopsin versus Trichomes mg^{-1} (x 10^{-3}) conversion in Figure 4; data need to be digitized
Microcystin (MC)	*Planktothrix agardhii*	England; Turkey	29.4 (2.3) – 34.9 (3.7) pg $filament^{-1}$; 0.2 (0.06) – 1.1 (0.6) fg μm^{-3} biovolume	Akcaalan et al. (2006)	
	Planktothrix agardhii	55 German lakes	1,500 – 2,200 µg g^{-1} dry weight; 0.25 – 0.5 µg MC µg chlorophyll a^{-1}	Fastner et al. (1999)	derived from Figure 2
	Planktothrix rubescens	England; Turkey	28.2 (7.1) – 53.6 (20.6) pg $filament^{-1}$; 0.9 – 3.4 (1) fg μm^{-3}	Akcaalan et al. (2006)	
	Planktothrix rubescens	55 German lakes	1,600 – 4,000 µg g^{-1} dry weight; 0.22 – 0.5 µg MC µg chlorophyll a^{-1}	Fastner et al. (1999)	derived from Figure 2
	Planktothrix rubescens	Italy lake		Salmaso et al. (2014)	has regression formulas for both MC versus chlorophyll and MC versus biovolume
	Planktothrix spp.		0.38 – 6.01 µg mg dry $weight^{-1}$ 0.17 – 4.5 µg mm^{-3} biovolume	Kurmayer et al. (2016)	low and high MC producing strains of *P. rubescens* and *P. agardhii*

Toxin	Species	Site/Clone	Conversion	Reference	Notes
Microcystin (continued)	*Planktothrix* spp.	UTEX 2388	87.9 (5.2) – 339 μg MC-LR g^{-1} dry weight; 0.56 (0.03) – 2.47 (0.03) mg MC-LR g^{-1} protein; 467 (8.1) – 773.5 g MC-RR g^{-1} dry weight; 3.00 (0.05) – 5.63 (0.03) mg MC-LR g^{-1} protein	Oh et al. (2000)	has values for MC-LR and MC-RR
	Microcystis aeruginosa	Portugal lake		Vasconcelos et al. (2011)	has cell versus MC concentration in Figure 4
	Microcystis aeruginosa	UTEX 2388	$y = 0.661x - 38.9$ ($r^2 = 0.569$); y: (MC μg g^{-1}), x: (chlorophyll *a* μg L^{-1})	Lee et al. (2000)	
	Microcystis aeruginosa	France lake		Sabart et al. (2010)	has MC versus cell concentrations in Figures 2 and 3
	Microcystis aeruginosa	Lake Biwam, Japan		Ozawa et al. (2005)	has MC versus cell concentration, chlorophyll *a* in Figure 2
	Microcystis aeruginosa	MASH01-A19	1.2 – 9.3 mg g^{-1} dry weight	Orr and Jones (1998)	estimated from Figure 4
	Microcystis spp.	San Francisco estuary	0 – 1 μg g^{-1} dry weight	Lehman et al. (2008)	derived from Figure 6
	Microcystis spp.	55 German lakes	0 – 1000 μg g^{-1} dry weight 0.08 – 0.31 μg MC μg chlorophyll a^{-1}	Fastner et al. (1999)	derived from Figure 2; derived from Figure 7
	Microcystis spp.	Lake Suwa, Japan	1.20 – 136 μg MC-RR 100 mg^{-1} dry weight; 4 – 89.8 μg MC-RR 100 mg^{-1} dry weight;	Park et al. (1998)	estimated from Figure 7
	Unknown	Quebec, Canada		Giani et al. (2005)	has biomass (g C L^{-1}) versus MC (mg g^{-1}) in Figure 4

Appendix F References

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APPENDIX G. TABLES OF STATE-ISSUED GUIDELINE SPECIFIC TO ANIMAL CYANOTOXIN POISONING

G.1 California

Table G-1. California Environmental Protection Agency (2012) Action levels for Selected Pet and Livestock Scenarios

	Microcystins[a]	Cylindrospermopsin	Media (units)
Subchronic water intake, dog[b]	2	10	water (µg/L)
Subchronic crust and mat intake, dog	0.01	0.04	crusts and mats (mg/kg dw)[c]
Acute water intake, dog[d]	100	200	water (µg/L)
Acute crust and mat intake, dog	0.5	0.5	crusts and mats (mg/kg dw)[c]
Subchronic water intake, cattle[e]	0.9	5	water (µg/L)
Subchronic crust and mat intake, cattle[e]	0.1	0.4	crusts and mats (mg/kg dw)[c]
Acute water intake, cattle[e]	50	60	water (µg/L)
Acute crust and mat intake, cattle[e]	5	5	crusts and mats (mg/kg dw)[c]

[a] Microcystins LA, LR, RR, and YR all had the same RfD so the action levels are the same.
[b] Subchronic refers to exposures over multiple days.
[c] Based on sample dry weight (dw).
[d] Acute refers to exposures in a single day.
[e] Based on small breed dairy cows because their potential exposure to cyanotoxins is greatest.

Table G-2. California Environmental Protection Agency (2012) Reference Doses and Acute and Subchronic Action Levels for Canine Exposure to Cyanotoxins in Drinking Water

	Microcystin	Cylindrospermopsin
Water consumption L/kg-d	0.085	0.085
Uncertainty factor (unitless)	3	3
Acute RfD[a] mg/kg/d	0.037	0.04
Acute action level µg/L	100	200
Subchronic RfD mg/kg/d	0.00064	0.0033
Subchronic action level µg/L	2	10

Reference:

Butler N, Carlisle J, Kaley KB, & Linville R (2012). Toxicological Summary and Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins. http://www.waterboards.ca.gov/water_issues/programs/peer_review/docs/calif_cyanotoxins/cyanotoxins053112.pdf. Last Accessed: 08/03/2016.

G.2 Oregon

Table G-3. Oregon Dog-specific Guideline Values for Cyanotoxins in Recreational Waters (µg/L)

	Microcystin	Cylindrospermopsin
Dog Guidance Value	0.2	0.4

Note: All dog-specific guideline values have been changed in this revision because California EPA's estimate of the amount of water an exercising dog consumes per kilogram body weight was updated in 2012 (from 0.168 to 0.255 L/kg-day). Current dog-specific guideline values are now consistent with the California EPA update. The dog-specific value for saxitoxins was further modified by application of an uncertainty factor to the dog-specific TDI for interspecies differences in sensitivity between humans (the species in the critical study) and dogs.

Reference:

Oregon Health Authority (2016). Oregon Harmful Algae Bloom Surveillance (HABS) Program Public Health Advisory Guidelines: Harmful Algae Blooms in Freshwater Bodies. https://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Documents/HABPublicHealthAdvisoryGuidelines.pdf.

G.3 Grayson County, Texas

Table G-4. Grayson County Texas Microcystin Guidelines for Dogs

Quantity of Lake Water Ingested to Receive a Potentially Lethal Dose of Microcystin, Assuming that Mouse and Dog Toxic Responses are Equivalent

	Gallons of Water	*Pounds of Water*
10 pound dog	2.70	22.50
80 pound dog	21.57	180.00

Quantity of Lake Water Ingested to Receive a Potentially Lethal Dose of Microcystin, Assuming that Mouse and Dog Toxic Responses are Equivalent (at actual concentrations found in Grand Lake, Oklahoma, in June 2011)
Highest measured concentration of Microcystin was 358 ppb.

	Gallons of Water	*Pounds of Water*
10 pound dog	0.15 (19.3 ounces)	1.26
80 pound dog	1.21	10.06

*This is not including additional dose amounts that could be ingested from a dog self grooming algae scum off its fur.
**LD50 for Microcystin- mouse used in Calculations = 45 mcg/kg
***20 ppb Microcystin is algal toxin threshold for BGA Warning (condition red)

Quantity of Lake Water Ingested to Receive a Potentially Lethal Dose of Cylindrospermopsin, Assuming that Mouse and Dog Toxic Responses are Equivalent
20 ppb Cylindrospermopsin in Lake Water

	Gallons of Water	*Pounds of Water*
10 pound dog	263	2200
80 pound dog	2109	17601

*This is not including additional dose amounts that could be ingested from a dog self grooming algae scum off its fur.
**LD50 for Cylindrospermopsin- mouse used in Calculations = 4400 mcg/kg
***20 ppb Cylindrospermopsin is algal toxin threshold for BGA Warning (condition red)

Reference:
Lillis J, Ortez A, & Teel JH (2012). *Blue-Green Algae Response Strategy*. Sherman, Texas. http://www.co.grayson.tx.us/users/Health_Dept/Docs/Blue-Green_Algae_Response_Strategy.pdf