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

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NOTICES

This document has been drafted and approved for publication by the Health and Ecological Criteria Division, Office of Science and Technology, United States Environmental Protection Agency, and is approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Section 304(a) of the Clean Water Act (CWA) requires the Administrator of the U.S. Environmental Protection Agency (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.

The 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. The 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. Alternatively, states may consider using these same values when adopting new or revised water quality standards (WQS). If adopted by states as WQS and approved by the 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.

This document has undergone an EPA intra-agency peer-review process. The Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency has completed the final review and the document is approved for publication. The values were derived using the existing peer-reviewed and published science on the adverse human health effects of the toxins including previous EPA analysis, such as the EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* and *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (HESDs), and the 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, 2015b, 2015c, 2015d). The EPA used established criteria methodologies (U.S. EPA 2000) and recreation-specific exposure parameters from the EPA's *Exposure Factors Handbook* (EFH) (U.S. EPA 2011) to derive these values. Detailed information that can be found in the EPA's HESDs and Drinking Water Health Advisories is summarized in this document.

The term "water quality criteria" is used in two sections of the CWA section 304(a)(1) and section 303(c)(2). The term has a different legal meaning 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 the state or authorized tribe adopts water quality criteria associated with specific designated uses as WQS under section 303, and approved by the EPA, they become applicable CWA WQS in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal WQS could have the same numerical values as criteria developed by the EPA 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. States and tribes can adopt criteria into their standards. When approved by the EPA, the criteria become Clean Water Act-applicable WQS. Guidelines to assist in modifying the criteria recommendations presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 2012).

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 CWA and EPA regulations on the basis of specific facts presented and scientific information then available.

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The EPA gratefully acknowledges the valuable contributions of the EPA internal technical reviewers who reviewed this document. Staff from the following EPA program and regional offices completed a formal review of these *Human Health Recreational Ambient Water Quality Criteria (AWQC) or Swimming Advisories for Microcystins and Cylindrospermopsin*.

U.S. EPA Office of Children's Health Protection

U.S. EPA Office of General Counsel

U.S. EPA Office of Policy

U.S. EPA Office of Research and Development

U.S. EPA Office of Water

Office of Ground Water and Drinking Water

Office of Science and Technology

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ACRONYMS AND ABBREVIATIONS

ACC	ambient cyanotoxin concentration
AWQC	ambient water quality criteria
AWWARF	American Water Works Association Research Foundation
BAX	BCL2 Associated X, Apoptosis Regulator
Bcl-2	BCL2 Apoptosis Regulator
BGAS	blue-green algae supplements
BID	BH3 interacting domain death agonist
BW	body weight
C.	*Cylindrospermopsis*
CalEPA	California Environmental Protection Agency
CAS	Chemical Abstracts Service
CAWS	Chicago Area Waterway System
CCD	cyanobacterial cell density
CDC	U.S. Centers for Disease Control and Prevention
CDEEP	Connecticut Energy and Environmental Protection
CDPH	Connecticut Department of Public Health
CFU	colony forming unit
CI	confidence interval
cm	centimeter
CTA	cell toxin amount
CWA	Clean Water Act
CyAN	Cyanobacteria Assessment Network
CYP450	Cytochrome P450
DFB	DeFlorio-Barker et al. (2017)
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
DON	dissolved organic nitrogen
dw	dry weight
E.	*Escherichia*
EFH	*Exposure Factors Handbook*
ELISA	Enzyme Linked Immunosorbent Assay
EPA	U.S. Environmental Protection Agency
FAQs	frequently asked questions
Fe	iron
fg	femtogram
g	grams
GI	gastrointestinal
GI2	more severe gastrointestinal symptom index
GM	geometric mean
GSD	geometric standard deviation

HAB	harmful algal bloom
HABISS	Harmful Algal Bloom-related Illness Surveillance System
HESD	Health Effects Support Document
HPLC	high performance liquid chromatography
IDEQ	Idaho Department of Environmental Quality
IQR	interquartile range
IR	ingestion rate
kg	kilograms
km	kilometer
K_{oc}	soil organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LA	leucine, alanine
LC/MS/MS	liquid chromatography with tandem mass spectrometry
LF	leucine, phenylalanine
LC_{50}	lethal concentration causing the death of 50 percent of a group of test animals
LD_{50}	lethal dose causing the death of 50 percent of a group of test animals
LOAEL	lowest-observed-adverse-effect-level
LOD	level of detection
LPS	lipopolysaccharide
LR	leucine, arginine
LW	leucine, tryptophan
LY	leucine, tyrosine
M.	*Microcystis*
m^3	cubic meter
mcy	three-letter nomenclature for genes that produce microcystins
mg	milligram
mL	milliliter
MW	molecular weight
MS	mass spectroscopy
n	sample size
N	nitrogen
N/A	not available
NASA	National Aeronautics and Space Administration
ng	nanogram
NHMRC	National Health and Medical Research Council
NLA	National Lakes Assessment
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no-observed-adverse-effect-level
NORS	National Outbreak Reporting System
NYSDOH	New York State Department of Health

OATP	organic anion transporting polypeptide
OHHABS	One Health Harmful Algal Bloom System
OPP	EPA Office of Pesticide Programs
OR	odds ratio
ORSANCO	Ohio River Valley Water Sanitation Commission
P	phosphorus
PCR	polymerase chain reaction
pg	picogram
pH	potential of hydrogen
ppb	parts per billion
PWS	public drinking water system
qPCR	quantitative polymerase chain reaction
rDNA	ribosomal deoxyribonucleic acid
RfD	reference dose
ROS	reactive oxygen species
RR	relative risk or when microcystin-RR it means arginine, arginine
RSC	relative source contribution
SDWA	Safe Drinking Water Act
SWIMODEL	Swimmers Exposure Assessment Model
t	event duration
TBD	to be determined
TDI	tolerable daily intake
TN:TP	total nitrogen ratio to total phosphorus
TOXLINE	Toxicology Literature Online
U.S.	United States of America
UF	uncertainty factor
URL	Uniform Resource Locator
µg	microgram
μm^3	cubic micrometer
USGS	U.S. Geological Survey
WHO	World Health Organization
WHOI	Woods Hole Oceanographic Institute
WoS	Web of Science
WQS	water quality standards
WSDE	Washington State Department of Ecology
YR	tyrosine, arginine

1.0 EXECUTIVE SUMMARY

Cyanobacteria, also commonly referred to as blue-green algae, are photosynthetic bacteria that are ubiquitous in nature and are found in 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. Nitrogen and phosphorus levels, the ratio of nitrogen to phosphorus, water temperature, organic matter availability, light attenuation, pH, and water column stratification are environmental factors that play an important role in the development of cyanobacterial blooms and their production of cyanotoxins. Some cyanobacteria, but not all, have the ability to produce toxins. The toxin-producing cyanobacteria contain genes that confer the ability to produce toxins and are referred to as toxigenic cells. The abundance of toxigenic cyanobacteria can vary within the overall cyanobacteria population, between waterbody to waterbody, and over time within a single waterbody.

Microcystins can be produced by a variety of toxigenic cyanobacteria genera, including *Microcystis*, *Anabaena*, *Dolichospermum, 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 featuring leucine and arginine (microcystin-LR), which is also a frequently monitored congener. Microcystins are water soluble and tend to remain contained within the toxigenic cyanobacterial cell until the cell breaks and they are released into the water. Microcystins typically have a half-life of four to 14 days in surface waters or may persist longer, depending on factors such as photodegradation, bacteria, and the presence of organic matter. Microcystins can persist even after a toxigenic cyanobacterial bloom is no longer visible.

Cylindrospermopsin can be produced by a variety of toxigenic cyanobacteria species, including *Cylindrospermopsis raciborskii*, *Aphanizomenon*, *Anabaena*, *Lyngbya wollei*, and *Raphidiopsis*. Some of these species tend not to form visible surface scums, and the highest concentrations of total cyanobacterial cells typically occur below the water surface. Two congeners of cylindrospermopsin, as well as two structural analogs, have been identified. Cylindrospermopsin can 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 eight weeks have been reported for cylindrospermopsin in surface waters.

This document for microcystins and cylindrospermopsin focuses on the human health risks associated with incidental ingestion while recreating in freshwaters containing these harmful cyanotoxins. The recommended cyanotoxin values apply to freshwaters with the recreational designated use. The toxins that are produced by cyanobacteria growing in freshwaters can enter estuarine and marine waters as waters containing the toxins flow downstream. The EPA recognizes that there may be circumstances where harmful cyanobacterial blooms (also known as harmful algal blooms or HABs) can impact downstream marine and estuarine waters. This document provides information on occurrence and incidental ingestion in estuarine and marine waters for states to consider but does not provide recommendations for those waters. 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. This document does not address or provide recommendations for non-recreational exposures.

The EPA is publishing these recommended values for microcystins[1] and cylindrospermopsin under the Clean Water Act (CWA) section 304(a) for states to consider as the basis for swimming advisories for notification purposes to protect public health in recreational waters. The 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. Alternatively, states may consider using these same values when adopting new or revised water quality standards (WQS). If adopted as WQS and approved by the EPA under the CWA section 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.

The recommended values in this document leverage the information that the EPA collected and evaluated in its *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* and *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (HESDs), and the 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, 2015b, 2015c, 2015d).

The 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* (U.S. EPA 2015d). Exposure to elevated levels of microcystins can potentially lead to liver damage. The critical study for the derivation of the microcystins RfD was conducted by Heinze (1999) based on rat exposure to microcystin-LR in drinking water. The critical effect from this study was slight to moderate liver lesions with necrosis and increased liver weight and enzymes associated with tissue damage. The EPA established the RfD based on microcystin-LR and used it as a surrogate for other microcystin congeners. Monitoring and toxicity studies suggest that the microcystin-LR is the most frequently occurring congener and is more toxic than other congeners of microcystin evaluated (Loftin et al. 2016b; U.S. EPA 2015d; Ito et al. 2002; Rinehart et al. 1994; Vesterkvist and Meriluoto 2003; WHO 1999). The EPA used the RfD to derive its previously published Drinking Water Health Advisories for microcystins (U.S. EPA 2015a) and the recommended values in this document. The dose and critical effects that the EPA used from Heinze (1999) to establish the RfD are supported by a Guzman and Solter (1999) study, also conducted in rats.

The EPA evaluated the health effects of cylindrospermopsin and derived an RfD in its 2015 *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA 2015c). The kidneys and liver appear to be the primary target organs for cylindrospermopsin toxicity. The critical study that the EPA used to derive the cylindrospermopsin RfD was conducted by Humpage and Falconer (2002, 2003) based on drinking water exposure to mice. Adverse effects on the kidneys were manifested by decreases in urinary protein concentration and increased relative kidney weight. Upon considering all effects observed by Humpage and Falconer (2002, 2003), increased relative kidney weight was considered the most appropriate basis for quantitation (U.S. EPA 2015c). The EPA used the RfD to derive its previously published Drinking Water Health Advisories for cylindrospermopsin (U.S. EPA 2015b).

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

[1] Microcystins comprise a class of over 100 congeners and unless specified otherwise, "microcystins" refers to total microcystins.

- For microcystins, the recommended recreational value is 8 micrograms (μg)/liter (L).
- For cylindrospermopsin, the recommended recreational value is 15 μg/L.

These values are based on the exposure experienced by recreating children due to their higher exposures compared with other age groups. Given that toxigenic cyanobacterial blooms typically are seasonal events, recreational exposures are likely to be episodic, and may be short term in nature. The EPA recommends that if used as a swimming advisory to protect swimmers at a beach, these values not be exceeded on any single day. If used as a water quality criterion for assessment and listing purposes, the EPA recommends a maximum of three excursions across a recreational season and observation of that pattern across multiple years to reflect seasonal dynamics and occurrence patterns of HABs.

At this time, available data are insufficient to develop quantitative recreational values for total cyanobacterial cell density related to inflammatory health endpoints. The reported epidemiological relationships between cell density exposure and specific health outcomes (e.g., dermal symptoms, eye/ear irritation, fever, gastrointestinal (GI) illness, and respiratory symptoms) are not consistent. The uncertainties related to the epidemiological study differences, such as study size, species and strains of cyanobacteria present, and the total cyanobacterial cell densities associated with significant health effects, do not provide sufficient information to determine a consistent association between total cyanobacterial densities associated with adverse inflammatory health effects. The EPA recognizes that some states have included total cyanobacterial cell density values as an important part of their HAB management strategy. Available information on health endpoints, cell density, and guidelines developed by other authorities on total cyanobacteria cells is described in the Effects Characterization section of the document (section 7.5) and in Appendix D.

Because the EPA's recommendations in this document are cyanotoxin concentrations, it can be helpful for risk-management purposes to understand how this relates to toxigenic cyanobacteria in the waterbody, as the abundance of toxigenic cells in a water body affects the amount of cyanotoxin produced. The number of toxigenic cyanobacteria relative to the number of total cyanobacteria can vary in time and space. Quantifying the abundance of toxigenic cyanobacteria is a better predictor of potential toxin production compared to quantifying total cyanobacteria. The EPA presents a toxigenic cell number based on the number of toxigenic cells that could produce microcystins equivalent to the recommended magnitude. The Effects Characterization section also describes gene-based detection methods (i.e., quantitative polymerase chain reaction (qPCR)) that can target and quantify the toxigenic subpopulation of cyanobacteria that are present in a waterbody.

2.0 INTRODUCTION AND BACKGROUND

Section 304(a) of the CWA requires the Administrator of the 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.

Currently there are no U.S. federal water quality criteria or regulations for cyanobacteria or cyanotoxins in drinking water under the Safe Drinking Water Act (SDWA) or in ambient waters under the CWA. No cyanotoxins are included on EPA's priority pollutant list.[2] In 2015, the EPA published non-regulatory Drinking Water Health Advisories (U.S. EPA 2015a, 2015b) to provide information for public health officials or other interested groups on two cyanotoxins (microcystins and cylindrospermopsin) that can affect drinking water quality but are not regulated under SDWA.

The EPA is publishing these recommended values for microcystins and cylindrospermopsin under the CWA section 304(a) for states to consider as the basis for swimming advisories for notification purposes to protect public health in recreational waters. The 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. Alternatively, states may consider using these same values when adopting new or revised WQS. If adopted as WQS and approved by the EPA under the CWA section 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.

The EPA-recommended values in this document leverage the information that the EPA collected and evaluated in its *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* and *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (HESDs), and its *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, 2015b, 2015c, 2015d).

This document for microcystins and cylindrospermopsin focuses on the human health risks associated with incidental ingestion while recreating in freshwaters containing these harmful cyanotoxins. The recommended cyanotoxin values apply to freshwaters with the recreational designated use. The toxins that are produced by cyanobacteria growing in freshwaters can enter estuarine and marine waters as waters containing the toxins flow downstream. The EPA recognizes that there may be circumstances where harmful cyanobacterial blooms (also known as HABs) can impact downstream marine and estuarine waters. This document provides information on occurrence and incidental ingestion in estuarine and marine waters for states to consider but does not provide recommendations for those waters. 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. This document does not address or provide recommendations for non-recreational exposures.

International and State Guidelines

The World Health Organization (WHO 2003a) published a series of guideline values for recreational exposure to cyanobacteria associated with incremental severity and probability of health effects at increasing densities of total cyanobacteria and corresponding concentrations of chlorophyll *a* (if

[2] https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf

cyanobacteria dominate) (Table 2-1). The WHO also considered the potential for liver damage by microcystins in deriving the recommended total cyanobacterial cell densities. Potential concentrations of microcystins that could be associated with each guidance level are discussed in the WHO document. However, it should be noted that actual microcystin concentrations at each WHO action level could vary depending on the composition of toxigenic strains in the cyanobacterial community present and the dominant species of microcystin producer present in a bloom. For example, at a total cyanobacterial cell density of 100,000 cells/milliliter (mL), an estimated microcystin concentration of 20 µg/L could occur assuming all cells present are toxin-producing *Microcystis* species and the average cellular toxin content was 0.2 picogram (pg) microcystin per cell (WHO 2003a). Microcystin concentrations could range from 50 to 100 µg/L, or higher, if another toxin-producing species, such as *Planktothrix*, is present at the same cell density.

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

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Chlorophyll *a* (µg/L)	Estimated Corresponding Microcystin Levels (µg/L)
Low	≤ 20,000	≤ 10	< 10[a]
Moderate	> 20,000–100,000	> 10–50	2–4 to 20[a,b]
High	> 100,000	> 50	> 20

[a] WHO estimated that 2 to 4 µg microcystins/L may be expected, with 10 µg/L possible, at a cell density of 20,000 cells/mL if microcystin-producing cyanobacteria are dominant.

[b] At 100,000 cyanobacterial cells/mL, a concentration of 20 µg microcystins/L is likely if the bloom consists of *Microcystis* and has an average toxin content of 0.2 pg/cell.

For these guidelines, the WHO recommended values that included the potential health effects from exposure to total 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 2003a). The different guideline levels were an effort to distinguish between irritative or inflammatory-response symptoms associated with total cyanobacterial cells and the more severe hazard of exposure to elevated concentrations of cyanotoxins, particularly microcystins. The cell-associated inflammatory responses are represented by the low probability of adverse health effects category of ≤ 20,000 cells/mL, corresponding to ≤ 10 µg/L chlorophyll *a* if cyanobacteria dominate. 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 WHO high-risk category includes both > 100,000 cells/mL, corresponding to 50 µg/L of chlorophyll *a*, if cyanobacteria dominate, and > 20 µg/L microcystin levels. Health effects at this level are expected to be primarily due to the toxic effects of microcystins. Very high densities of cells occurring in scums—for example, > 10 million cells/mL or > 5,000 chlorophyll *a*—can be associated with very high concentrations of toxin, for example 2,000 µg/L of microcystins in the top 4 cm of a water body (WHO 2003a). Scums that accumulate along the shoreline due to wind can be associated with a thousand-fold higher density of cells (WHO 2003a).

The WHO guideline value development was informed by results from a review conducted by Chorus and Bartram (1999). A primary study identified in this review was a prospective epidemiology study by Pilotto et al. (1997), which evaluated health effects after recreational exposure to total cyanobacteria and reported associations between total cyanobacterial cell densities and health. Pilotto et al. (1997) found a

significant association among recreators exposed to greater than 5,000 cells/mL. The 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 2003a). While the association among recreators exposed to greater than 5,000 cells/mL for more than one hour and one or more symptoms reported in Pilotto et al. (1997) was statistically significant, the WHO states that they represented less than 30 percent of the individuals exposed (Chorus and Bartram 1999). Therefore, the level of health effect and the small number of people affected at 5,000 cells/mL were not considered by the WHO to be a basis to justify action (WHO 2003a).

The WHO pointed out that the potential concentration of microcystins could vary depending on the composition of toxigenic strains within the overall cyanobacterial community present and the dominant species of microcystin producer present in a bloom. The WHO states that, at the same cyanobacterial cell density, cyanotoxin levels could approximately double if *Planktothrix agardhii* were the dominant member of the community.

Many countries have adopted the multiple parameters that the WHO discusses for recreational waters including cell density, biovolume, and cyanotoxin concentration (see Table 2-2). Some international authorities have multiple action levels. For brevity, Table 2-2 presents the guideline reflecting the lowest concentration of microcystins or density of cyanobacterial cells or narrative guidelines that recommended or triggered a health protective action for countries that have adopted action levels. For a more complete list of guideline or action levels and recommended actions for international jurisdictions, see Appendix A. The EPA did not identify any recreational guideline levels for cylindrospermopsin established by other international regulatory authorities.

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): $\underline{\geq}$ 10 µg/L or *Microcystis aeruginosa* (total): $\underline{\geq}$ 500 to < 5,000 cells/mL or cyanobacteria (total): $\underline{\geq}$ 0.4 to < 4 mm^3/L (where a known toxin producer is dominant in the total biovolume) or total biovolume of all cyanobacterial material $\underline{\geq}$ 10 mm^3/L (where known toxins are not present)	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]

Jurisdiction	Lowest Recreational Water Guideline/Action Level[a]	Reference
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: ≥ 1 mm^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]	microcystin-LR: > 20 µg/L equivalents or cyanobacterial cell count for cyanotoxin-producing species other than those that produce microcystins (e.g., cylindrospermopsin) > 100,000 cells/mL (± 20 percent) or transparency ≤ 1 m and total phosphorus > 20 µg/L and total cyanobacterial cell count > 2,000 to ≤ 20,000 cells/mL (± 20 percent) or transparency ≥ 1 m and total phosphorus > 20 µg/L and total cyanobacterial cell count ≤ 2,000 cells/mL	Funari et al. (2017)
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)
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 microcystins/L, assuming cyanobacteria dominance)	Chorus and Bartram (1999); WHO (2003a)

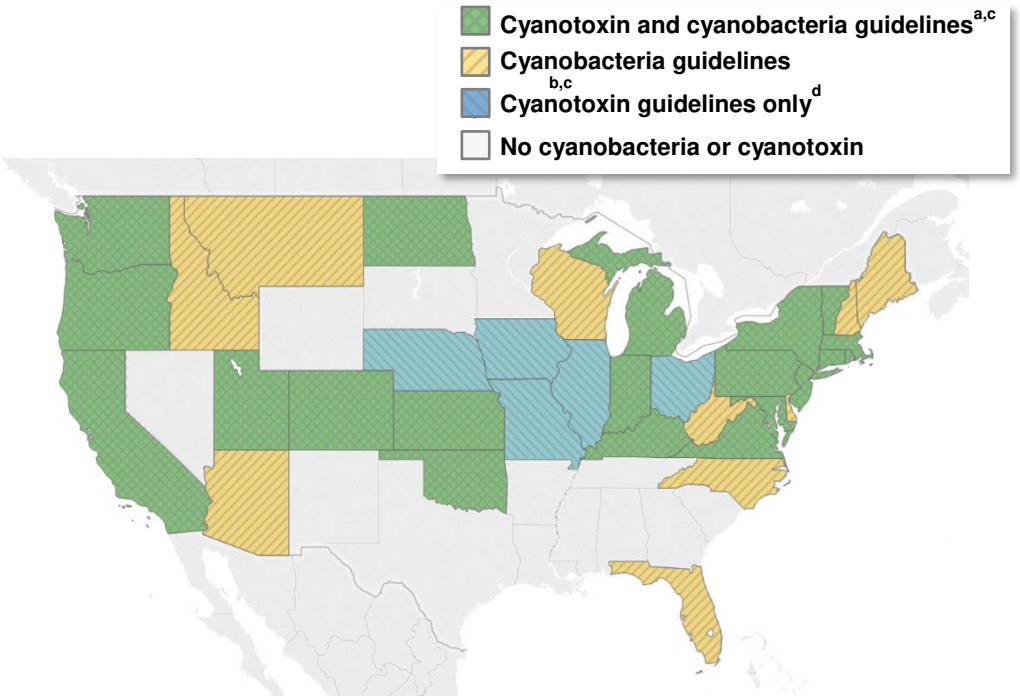
[a] More details are provided in Appendix A.

[b] The lowest guideline values for each quantitative parameter (i.e., cyanotoxin concentration, cyanobacterial cell density, biovolume) 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.

As of March 2018, approximately 35 U.S. states have implemented cyanobacterial HAB guidelines for recreational waterways. As graphically shown in Figure 2-1, five states have quantitative or qualitative cyanotoxin guidelines only, and 20 states have quantitative guidelines for cyanotoxins, as well as either quantitative or qualitative guidelines for total cyanobacterial cell density. Qualitative guidelines for cell density use visual inspection rather than quantitative detection methods. In addition, 10 states had quantitative guidelines for cyanobacterial cell density only or had qualitative guidelines for cyanobacteria only. Seven states have guideline levels that address toxin-producing cyanobacteria as a proportion of the total cyanobacterial population or include a toxin-specific cyanobacteria cell density (California, Idaho, Maryland, New York, New Hampshire, Oregon, and Virginia). The Karuk Tribe, located in California, developed cell-based values for posting cyanotoxin public health warnings for the tribe's recreational waters (Kann 2014). Its values were based on the site-specific relationships between the cell densities of *Microcystis* and the level of microcystins observed in Karuk waters. For example, in the Klamath River, at 20,000 cells *Microcystis*/mL, the probability of exceeding 4 µg/L microcystins was 55 percent, while at 5,000 cells/mL there were no exceedances. Because the probability of exceeding the microcystins benchmark rapidly increased at cell densities above 5,000 *Microcystis*/mL, the Karuk Tribe uses that value to inform decision-making for health warnings (Kann 2014).

Figure 2-1. 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] The EPA found that Texas and North Carolina published guidelines in the past, but the guidelines were no longer on their websites.

[d] Missouri has presence/absence testing for cyanotoxins and quantitative thresholds.

For brevity, Table 2-3 lists the lowest recreational water guideline or narrative guidelines or action levels for microcystins, cylindrospermopsin, or total cyanobacteria that trigger or recommend a health protective action for U.S. states. For a more complete list of state guideline or action levels see

Appendix B. Parameters and values used as the basis for guidelines varied across states, as did the methodologies for developing the values.

Table 2-3. State Guideline or Action Levels for Microcystins, 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); Cyanobacteria Harmful Algal Bloom Network (2016a, 2016b)
	Cylindrospermopsin: 1 µg/L	
	Toxin-producing cyanobacteria: 4,000 cells/mL	
	Site-specific indicators of cyanobacteria (e.g., blooms, scums, mats)	
Colorado	Microcystin-LR: ≥ 10 µg/L and < 20 µg/L	Colorado Department of Public Health and Environment (2016)
	Cylindrospermopsin: ≥ 7 µg/L	
	Potentially toxic algae are visible	
Connecticut[b]	Combination of visual inspection, cell counts: Visual rank category 2: Blue-green algae cells > 20,000 cells/mL and < 100,000 cells/mL	Connecticut Department of Public Health (CDPH) and Connecticut Energy and Environmental Protection (CDEEP) (CDPH and CDEEP 2017; CDEEP 2017)
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 (2019)
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 (2018); Illinois Environmental Protection Agency (2013)
Indiana	Blue-green algae: 100,000 cells/mL	Indiana Department of Environmental Management (2018)
	Microcystin-LR: 4 µg/L	
	Cylindrospermopsin: 8 µg/L	
Iowa	Microcystin: ≥ 20 µg/L	Iowa Environmental Council (2018)
Kansas	Cyanobacteria: ≥ 80,000 and < 250,000 cells/mL	Kansas Department of Health and Environment (2015a); Kansas Department of Health and Environment (2015b)
	Microcystin: ≥ 4 and < 20 µg/L	
Kentucky	Blue-green algae: > 100,000 cells/mL	Kentucky Department for Environmental Protection (2014)

State	Lowest Recreational Water Guideline or Action Level[a]	Reference
	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	Wazniak personal communication (2016); Maryland Department of Natural Resources (2014)
Massachusetts	Blue-green algae: > 50,000 cells/mL	Massachusetts Bureau of Environmental Health (2015); Massachusetts Department of Public Health (2008)
	Microcystins: ≥ 14 µg/L	
Michigan	Microcystin: ≥ 20 µg/L	Michigan Department of Environmental Quality (2018); Kohlhepp G (2015)
	Chlorophyll *a*: > 30 µg/L and visible surface accumulations/scum are present, or cells are visible throughout the water column	
Missouri	Microcystins: presence (test strip range 0 to 10 ng/mL)	Missouri Department of Natural Resources (2017)
	Cylindrospermopsin: presence (test strip range 0 to 10 ng/mL)	
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 (2018)
New Hampshire	Cyanobacteria: > 50 percent of total cell counts from toxigenic cyanobacteria	New Hampshire Department of Environmental Services (2014)
New Jersey	Microcystins (as total including -LR and other detectable congeners): 3 µg/L	New Jersey Department of Environmental Protection (2017)
	Cylindrospermopsin: 8 µg/L	
	Cyanobacterial cell count: ≥ 20,000 cells/mL	
New York	Bloom: credible report or digital imagery of a bloom determined as likely to be potentially toxic cyanobacteria by DEC or DOH staff	New York State Department of Environmental Conservation (2017)
	Blue-green chlorophyll *a*: > 25 µg/L	
	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	Microcystins: 6 µg/L	Ohio EPA (2016)
	Cylindrospermopsin: 5 µg/L	
Oklahoma	Cyanobacteria: 100,000 cell/mL	Oklahoma Legislature (2012)

State	Lowest Recreational Water Guideline or Action Level[a]	Reference
	Microcystin: > 20 µg/L	
Oregon	Cylindrospermopsin: ≥ 8 µg/L	Oregon Health Authority (2018)
	Microcystin: ≥ 4 µg/L	
	Microcystis: > 40,000 cells/mL	
	Planktothrix: > 40,000 cells/mL	
	Toxigenic species: > 100,000 cells/mL	
	Visible scum with documentation and testing	
Pennsylvania	Microcystin: > 6 µg/L	Pennsylvania Department of Environmental Protection (2014)
	Cylindrospermopsin: > 5 µg/L	
	HAB verified by visual observation	
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	
Utah	Cyanobacteria: 20,000–10,000,000 cells/mL	Utah Department of Environmental Quality and Department of Health (2017)
	Microcystin: 4–2,000 µg/L	
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 (2008); 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 and Human Resources (2015)
Wisconsin	Cyanobacteria: > 100,000 cells/mL	Wisconsin Department of Natural Resources (2012); Wisconsin Department of Health Services (2016)
	Visible scum layer	Werner and Masnado (2014) ; Wisconsin Department of Health Services (2016)

[a] More details are provided in Appendix B.
[b] Connecticut states "based on US EPA's draft recreational criterion, CT DPH suggests a cyanotoxin threshold of 4 µg/L microcystin."
[c] The EPA found that Texas published guidelines in the past, but the guidelines were no longer on its website.

3.0 NATURE OF THE STRESSORS

This section describes cyanobacteria and cyanobacterial blooms that have the potential to produce microcystins and cylindrospermopsin. It also describes the chemical and physical properties, sources and occurrence information in different media, environmental fate, and toxicokinetics for the cyanotoxins. The information in this section is based on information the EPA presented in its HESDs and Drinking Water Health Advisories (U.S. EPA 2015a, 2015b, 2015c, 2015d). The EPA conducted supplemental literature searches in September 2015 to capture new references related to the following topics:

- Levels of human exposure to cylindrospermopsin or microcystins through recreational water activities.
- Health effects for humans or animals exposed to cylindrospermopsin or microcystins.
- State and international safety levels or criteria for microcystins or cylindrospermopsin.
- Recreational exposure ingestion rates for children's age groups.
- Incidents of pet or livestock adverse health effects, including mortality, due to exposure to cyanotoxins.

For detailed information on these supplemental literature searches and the five research questions that correspond to the bullets above, see Appendix C.

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, referred to as cyanotoxins, which can adversely affect human health. Under the right conditions of water temperature, light, pH, nutrient availability, and other factors, cyanobacteria can reproduce rapidly, forming what are commonly referred to as cyanobacterial HABs. Other microorganisms can form HABs, but for the purpose of this document the usage of "HABs" refers to cyanobacterial HABs unless otherwise specified.

3.1 Cyanobacteria and Cyanobacterial Blooms

Cyanobacteria are photosynthetic prokaryotes (Seckbach and Oren 2007) and are ubiquitous in the environment. Cyanobacteria smaller than 2.0 μm are known as picocyanobacteria (Jakubowska and Szeląg-Wasielewska 2015). The chloroplast, found in photosynthetic eukaryotes like algae and plants, evolved from an endosymbiotic relationship with cyanobacteria (Kutschera and Niklas 2005). Ecologists historically grouped cyanobacteria, often referred to as "blue-green algae," with eukaryotic algae because they contain chlorophyll *a* and can 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, including picocyanobacteria, can produce bioactive compounds including toxins, which can be harmful. These biomolecules include hepatotoxic, neurotoxic, and cytotoxic compounds and compounds that can result in allergic reactions (Burkholder and Glibert 2006; Carmichael 1994; Jaiswal et al. 2008; Jakubowska and Szeląg-Wasielewska 2015; Śliwińska-Wilczewska et al. 2018; Volk and

Mundt 2007). Studies have shown that exposure to cyanobacterial cells can cause health effects that are independent of the cyanotoxins; this information is detailed in Appendix D.

Under certain conditions, cyanobacteria possessing the toxin synthesis genes, also referred to as toxigenic cyanobacteria, begin producing cyanotoxins. Numerous biotic and abiotic factors can influence not only the dominance of cyanobacteria within the overall phytoplankton community, but also the proportion of toxigenic cyanobacteria relative to non-toxin-producing cyanobacteria (Davis et al. 2009; Hyenstrand et al. 1998; McCarthy et al. 2009; Neilan et al. 2013; Gobler et al. 2016). Multiple species of cyanobacteria are capable of producing the same toxin, such as the microcystins, which can pose a risk to human and animal health (Crawford et al. 2017). Although scientists have observed a generalized relationship between cyanobacteria density or chlorophyll *a* and cyanotoxin concentration, these relationships are affected by the dominance of the toxin-producing cyanobacteria within the overall cyanobacterial community (Zhang et al. 2014; Loftin et al. 2016b).

Members of the genera *Microcystis*, *Dolichospermum* (*Anabaena*), *Nostoc*, *Fischerella*, *Planktothrix* (formerly *Oscillatoria*), and *Gloeotrichia* can produce microcystins (Carey et al. 2012b; Codd et al. 2005; Duy et al. 2000; Stewart et al. 2006c). *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. *Microcystis* 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). *Microcystis* have been documented throughout the United States (Carmichael 2001; Jacoby et al. 2000). Species of cyanobacteria, like *Microcystis*, that occur at or near the surface due to buoyancy and wind, can accumulate on shores and bays where they can form scums (Australian Government National Health and Medical Research Council 2008; WHO 2003b).

Cylindrospermopsin can be produced by a number of cyanobacterial species including *Raphidiopsis raciborskii* (formerly Cylindrospermopsis raciborskii),[3] *Aphanizomenon flos-aquae*, *Aphanizomenon gracile*, *Aphanizomenon ovalisporum*, *Umezakia natans*, *Anabaena bergii*, *Anabaena lapponica*, *Anabaena planctonica*, *Lyngbya wollei*, *Raphidiopsis curvata*, and *Raphidiopsis mediterranea* (B-Béres et al. 2015; Kokocinski et al. 2013; McGregor et al. 2011; Moreira et al. 2013). These species do not tend to form visible surface scums and the highest concentrations of cyanobacterial cells occurs below the water surface (Falconer 2005).

Cylindrospermopsin-producing cyanobacteria occur in tropical or subtropical regions, as well as warmer temperate regions. For example, *Cylindrospermopsis 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* (28.7 percent) (Burns 2008).

Research indicates that cyanotoxins can confer competitive advantage for survival and replication and are associated with physiological functions of cyanobacterial cell signaling, nutrient uptake, iron scavenging, maintenance of homeostasis, and protection against oxidative stress (Holland and Kinnear 2013). Cylindrospermopsin production provides a competitive advantage to cyanobacteria when phosphorus becomes scarce. Bar-Yosef et al. (2010) observed that when phosphorus is scarce, the

[3] Cyanobacteria taxonomy is continuously being revised. The genus *Cylindrospermopsis* has been renamed to *Raphidiopsis*. This document mostly maintains the genus name of *Cylindrospermopsis.*

cyanobacterium *Aphanizomenon ovalisporum* releases cylindrospermopsin, which causes other microorganisms to release alkaline phosphatase, a compound that will increase available free 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 ecological function of microcystins has not been determined conclusively (Zurawell et al. 2005). Studies comparing wild-types and mutants of a microcystin-producing 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). Gobler et al. (2007) observed decreased zooplankton grazing when toxigenic *Microcystis* were actively producing microcystin. Although cyanotoxins can negatively affect humans and other animals, research suggests that the primary functions of cyanotoxins are in cyanobacterial physiology and microbial ecology.

Cyanobacteria can regulate their buoyancy; thus, they can actively seek water depths with optimal growth conditions and will enlarge their gas vesicles to adapt to turbulent conditions. When weather conditions shift from turbulent to strongly stratified, excessively buoyant cells may accumulate at the surface because the regulation of buoyancy takes a few days (Australian Government National Health and Medical Research Council 2008, WHO 2003b). When the rate of cyanobacterial cell growth exceeds the loss rate for a population, positively buoyant, floating cyanobacterial cells can also accumulate at the surface (Falconer 1998). This accumulation can form a visibly colored scum on the water surface, which can contain more than 10,000 cells/mL (Falconer 1998). Scums can pose an elevated health risk to recreational users. The floating scum can be concentrated by prevailing winds in certain surface water areas, especially at the shore as is the case for *Microcystis*. Scums have frequently been reported to accumulate cells and cyanotoxin concentration by a factor of 1,000 or more, with million-fold accumulations resulting in pea soup consistency (Australian Government National Health and Medical Research Council 2008; WHO 2003b).

The microbial community can be complex and variable. It can consist of multiple different species and strains of cyanobacteria and other microbes. Microbial interactions can 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.

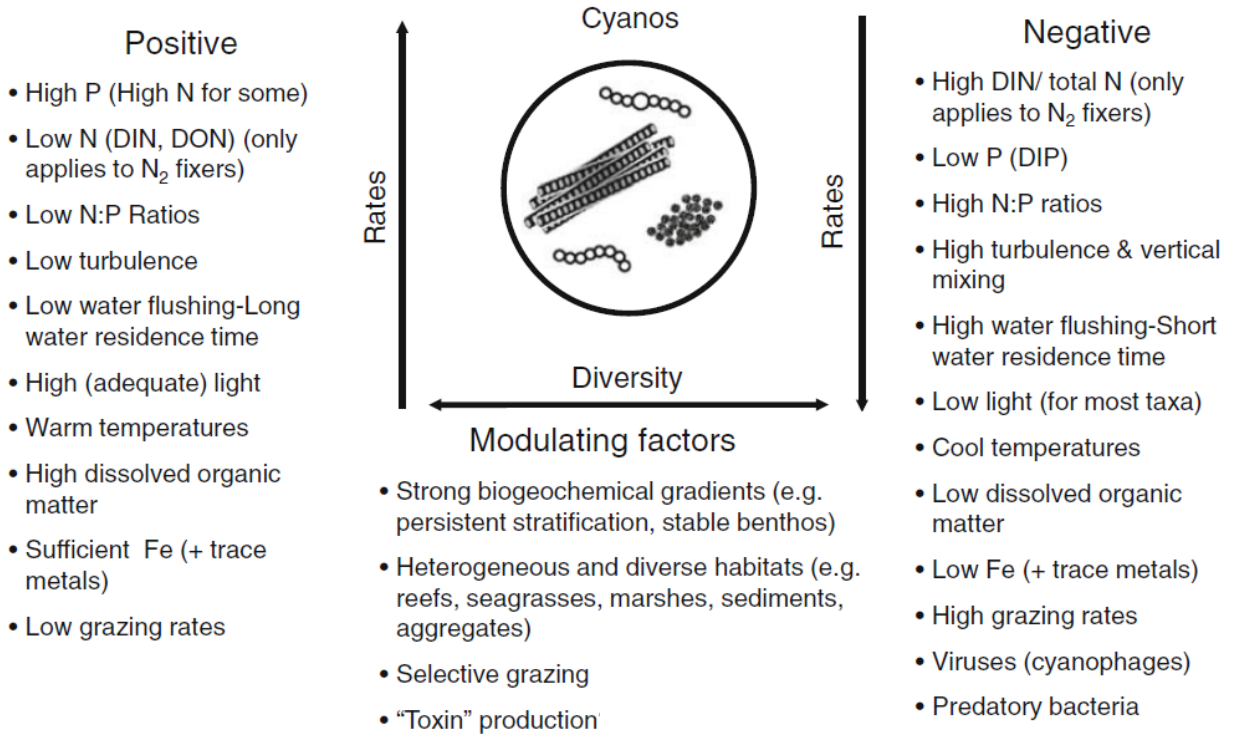
3.1.1 Environmental Factors Influencing Occurrence of Cyanobacteria and Cyanotoxins

A variety of physical, chemical, and environmental factors can influence both cyanobacteria proliferation and toxin production, including nutrient (e.g., nitrogen and phosphorus) concentrations, water temperature, light levels, and pH. Other factors include water turbulence, mixing, and flushing, oxidative stressors, and interactions with other biota (e.g., viruses, bacteria, and animal grazers), as well as their combined effects (Paerl and Otten 2013a, 2013b). See Figure 3-1.

Total 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 can vary among strains and clones of a single species (Carmichael 1994; Utkilen and Gjølme 1992) and within and between blooms (Codd and Bell 1985). Growth phase also can influence cyanotoxin production (Jaiswal et al. 2008). Biomass and toxin production do not necessarily coincide (section 7.5.2.3). Francy et al. (2016) modeled the relationship of

environmental variables compared to cyanotoxin levels. They demonstrated that some environmental factors such as measures of the algal community (e.g., phycocyanin, cyanobacterial biovolume, and cyanobacterial gene concentrations) and pH are strongly correlated with microcystin concentrations.

Figure 3-1. Environmental Factors Influencing Total Cyanobacterial Blooms, Reproduced from Paerl and Otten (2013b)



Cyanotoxins can be found inside the cell (i.e., intracellular) or external to the cell in the water (i.e., extracellular). The proportion of intracellular versus extracellular cyanotoxin can vary. Extracellular microcystins (either dissolved in water or bound to other materials) typically are 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 toxigenic cyanobacterial cells rupture or die. Cylindrospermopsin can 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 *Cylindrospermopsis raciborskii* released extracellularly (Griffiths and Saker 2003).

A complex interplay of environmental factors dictates the spatial and temporal changes in the concentration of cyanobacterial cells and their toxins with respect to the dominant species. 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 and Huisman 2008; Paerl and Otten 2013b).

Some cyanobacteria possess toxin genes that enable them to produce toxins, while other cyanobacteria do not contain toxin genes and therefore cannot produce toxins. For example, cyanobacteria that can produce microcystins contain a collection of genes, called "*mcy*" genes, that when expressed produce

microcystins. Multiple species of cyanobacteria can contain this set of genes. Together these species comprise microcystin-producing toxigenic cyanobacteria. Ten genes are in the microcystin gene cluster, *mcyA* through *mcyJ* (Tillett et al. 2000). Different researchers have studied the occurrence and prevalence of these genes within cyanobacteria populations.

Environmental factors can provide competitive advantages to *Microcystis* relative to other phytoplankton (Jacoby et al. 2000; Marmen et al. 2016). Evidence suggests that these environmental factors also affect the relative abundance of microcystin-producing strains and non-microcystin-producing strains (Marmen et al. 2016). *Microcystis* thrive 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, *Microcystis 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). The genetic composition of the bloom can also influence the degree of toxicity associated with an algal bloom. Lee et al. (2015) found that *Microcystis* typically comprised less than one percent of the total cyanobacterial abundance in Vancouver Lake, Washington, but the majority of the *Microcystis* cells contained the toxin-producing gene. Despite comprising a small percentage of the total cyanobacterial community in this lake, *Microcystis* were the sole microcystin-producing cyanobacteria and were responsible for microcystin concentrations that exceeded the WHO guidelines several times throughout the sampling period. In addition, increases in phosphate concentrations were associated with increases in both toxigenic and non-toxigenic *Microcystis* and with toxin production. The authors note that quantifying *Microcystis mcyE* gene (one of the genes responsible for toxin production) copy number, rather than relying solely on visual cell counts, might be a better estimate of overall cyanotoxin concentration (Lee et al. 2015).

Zhang et al. (2015) observed that low flow conditions favored total cyanobacteria and higher flow conditions favored green algae. 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).

Phytoplankton competition and food web interactions occur as blooms develop, persist, and decline, thereby impacting cyanotoxin concentrations in surface waters. In addition, 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 et al. 2011; Paerl and Huisman 2008; Paerl and Otten 2013b).

3.1.1.1 Nutrients

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 (Yang et al. 2016a; Beaulieu et al. 2013; Paerl et al. 2011). Cyanobacteria have been shown to dominate the phytoplankton communities in eutrophic lakes (Downing et al. 2001; Monchamp et al. 2014). Phosphorus loading has been linked to the proliferation of cyanobacteria and the shift toward cyanobacterial dominance of the phytoplankton community (O'Neil et al. 2012). However, it is important to consider both phosphorus and nitrogen when considering the occurrence of toxigenic cyanobacterial blooms. Cyanobacterial toxin concentrations are also associated with nutrient levels (Wang et al. 2002); however, different cyanobacteria species use organic and inorganic nutrient forms differently. Dolman et al. (2012) found that total cyanobacterial biomass was higher in lakes with above-

average nitrogen and phosphorus concentrations and that concentrations of all cyanotoxin groups were higher in lakes with higher total nitrogen and total phosphorus concentrations.

Paerl (2008) demonstrated that nitrogen and phosphorus additions, both independently and together, can stimulate primary productivity and *Cylindrospermopsis raciborskii* biomass. Elevated nitrogen and phosphorus loading can enhance the growth and cyanotoxin levels of *Microcystis* blooms and microcystin synthetase gene expression (Gobler et al. 2007; O'Neil et al. 2012; Marmen et al. 2016). Gobler et al. (2007) found that *Microcystis* dominance and toxin production was stimulated by elevated nitrogen and suppressed by nitrogen limiting conditions. Toxin production may cause the inhibition of grazing by mesozooplankton and further accumulation of cyanobacterial cells. Willis et al. (2015) found the highest growth rates for environmental isolates of *Cylindrospermopsis raciborskii* were observed with the addition of nitrogen.

The relative abundance of nitrogen and phosphorus can be an important consideration in regards to toxigenic cyanobacterial blooms. Loadings of nitrogen, or phosphorus, or both, to water bodies from agricultural, industrial, and urban sources influences the development of total cyanobacterial blooms and are associated with cyanotoxin production (Paerl et al. 2011). Smith (1983) was the first to describe a strong relationship between the relative amounts of nitrogen and phosphorus in surface waters and toxigenic 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* that produce toxins do not fix nitrogen. Many toxigenic cyanobacterial blooms are comprised of non-nitrogen-fixing genera and in the presence of elevated phosphorus, nitrogen can be a limiting factor for biomass proliferation and microcystin production (Gobler et al. 2007). Schindler et al. (2008) demonstrated that lower nitrogen inputs relative to phosphorus loadings can lead to dominance of nitrogen-fixing cyanobacteria in mesocosm- and ecosystem-scale experiments in prairie and boreal lakes. Otten et al. (2012) reported higher average microcystin concentrations and a higher prevalence of toxigenic *Microcystin* biomass at sites that had narrower TN:TP ratios (< 20) in Lake Taihu, China. Fortin et al. (2015) demonstrated that the dominance of *Microcystis* depended on the ratio of nitrogen to phosphorus, with a (mass) ratio 11:1 resulting in the highest abundance of *Microcystis*, whereas the concentrations of each nutrient were significant factors affecting the amount of biomass that could be generated.

Cyanotoxin concentration can be related to cyanobacterial cell abundance, which is facilitated by nutrient availability (Welker 2008), so nutrient concentration can be correlated to cyanotoxin concentration. Yuan et al. (2014; 2015) developed nutrient thresholds related to microcystin concentrations, cyanobacterial biovolume, and chlorophyll *a*. Nutrient availability, environmental conditions, and ecosystem interactions can affect the production and amount of toxins that cells produce and release (Bar-Yosef et al. 2010; Dolman et al. 2012; Graham et al. 2004; Paerl et al. 2001). For example, both nitrogen and phosphorus have been shown to promote the production of microcystins during bloom events (Davis et al. 2009; Gobler et al. 2016; Ha et al. 2009). Horst et al. (2014) found a significant positive relationship between cellular microcystin amounts and nitrate concentration with nitrogen limitation related to lower cell quotas of microcystin. Ha et al. (2009) found that microcystin concentrations were highly associated with *mcyA* gene copies and that high concentrations of nitrates and ammonium increased microcystin production by promoting the growth of toxigenic *Microcystis*. Elevated phosphorus has been shown to favor toxigenic strains over non-toxin strains coupled with higher intracellular toxin concentrations (Boopathi and Ki 2014; Burford et al. 2016).

Soluble phosphates and nitrates may also result in the increased production of microcystins (ILS 2000; O'Neil et al. 2012; Paerl and Scott 2010; Wang et al. 2010). Davis et al. (2009) found that growth rates

of toxigenic *Microcystis* were higher than nontoxic strains as temperature increased in the presence of elevated soluble phosphorus and that toxigenic cells contained more copies of the gene *mcyD* under these conditions. The authors conclude that lakes experiencing this combination of factors could experience more toxic blooms (Davis et al. 2009). In the Sacramento-San Joaquin delta in California nitrogen and phosphorus are available in non-limiting amounts and facilitate persistence of total cyanobacterial blooms (Berg and Sutula 2015). A study by Lehman et al. (2015) characterizes nitrogen sources of a *Microcystis* bloom in the San Francisco Estuary using stable isotopes. They reported that ammonium from the Sacramento River was the likely sole source of the nitrogen for most of the bloom, overriding nitrate contributions from the San Joaquin River.

Jacoby et al. (2000) characterized multiple physical and chemical environmental factors associated with blooms in the summer of 1994 and 1995 at Steilacoom Lake, Washington. The dominance of *Microcystis aeruginosa* in the lake was associated with low nitrogen-to-phosphorus ratios and low nitrate-nitrogen with sufficient ammonium-nitrogen. Microcystin concentrations were positively correlated with increasing soluble reactive phosphorus concentrations with the highest microcystin concentrations associated with a low ratio of soluble nitrogen to soluble reactive phosphorus (less than five). The authors reported that microcystin production per gram cyanobacterial biomass was not consistent, thus no relationship was found between *Microcystis aeruginosa* abundance and microcystin concentration. A significant positive relationship between total phosphorus concentrations and total cyanobacteria densities was observed in both years of the study (Jacoby et al. 2000).

During bloom events, nutrients on a local scale are incorporated into the production of biomass and decrease in the water column within the bloom, even in eutrophic water bodies. Kuniyoshi et al. (2013) showed that phosphate deficiency resulting from exponential biomass production can result in approximately seven-fold increase in microcystin synthesis. Bar-Yosef et al. (2010) reported that cylindrospermopsin-producing *Aphanizomenon* excrete cylindrospermopsin when phosphorus-limiting conditions occur within the bloom, to induce other cells to produce and excrete alkaline phosphatase, thus increasing availability of extracellular inorganic phosphate. Cylindrospermopsin is energetically cheaper for the cell to produce relative to alkaline phosphatase (Raven 2010) and coupled with a high-affinity phosphorus uptake protein also found in these cells, allows *Aphanizomenon* to increase rapidly, outcompeting other cyanobacteria and dominate a bloom (Bar-Yousef et al. 2010). Preußel et al. (2014) observed that cylindrospermopsin is actively released from *Aphanizomenon ovalisporum* cells subjected to phosphorus limitation, a condition that occurs during the exponential biomass production in a bloom event.

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 and Huisman 2008; Paerl and 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 suggest that reductions in nutrient concentration would reduce eutrophication and cyanobacterial bloom expansion. For example, analysis of observational data collected at high spatial scales support the idea that controlling total phosphorus and total nitrogen could reduce the frequency of high microcystin contamination events by reducing the biomass of total cyanobacteria in the system (Orihel et al. 2012; Scott et al. 2013; Yuan et al. 2014). In addition, reduction of phosphorus in the absence of concurrent

reductions in nitrogen loading may not effectively control the growth, toxicity, or both of cyanobacteria such as *Microcystis* (Gobler et al. 2016). Study authors concluded that reduction of specific nutrient species, such as soluble forms of nitrogen and phosphorus, could reduce the dominance of toxigenic cyanobacteria in the lake microbial community, which could, in turn, decrease the incidences of elevated toxin levels (Davis et al., 2010; Gobler et al. 2016).

3.1.1.2 Temperature

Cyanobacterial blooms commonly occur from spring to early fall in various regions of the United States (Wynne and Stumpf 2015). Conditions such as elevated water temperatures and increased vertical stratification in lakes and reservoirs can support proliferation of total cyanobacteria (Paerl and Huisman 2008). 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 and Adrian 2009; Weyhenmeyer 2001) suggest that an increase in temperature may influence cyanobacterial dominance in phytoplankton communities. Some cyanobacteria have higher optimal growth temperatures compared with other phytoplankton and can proliferate at higher water temperatures by outcompeting these other phytoplankton groups (Elliott 2010; Paerl et al. 2011). Warmer water temperatures favor surface bloom-forming cyanobacterial genera because they are heat-adapted, and their maximal growth rates occur at relatively high temperatures, with optimum growth temperatures ranging from 30 to 35°C and optimum microcystin production ranging from 20 to 25°C (Giannuzzi 2018; Reynolds 2006; Robarts and Zohary 1987; WHO 2003b). As the growth rates of the eukaryotic taxa decline in response to warming water temperature, cyanobacterial growth rates reach their optima. Davis et al. (2009) found in four U.S. lakes that concurrent increases in temperature and phosphorus concentrations yielded the highest growth rates of toxic *Microcystis* cells, which led them to conclude that eutrophication and warm temperatures may promote the growth of toxic, rather than nontoxic, populations of *Microcystis* leading to blooms with higher microcystin content.

Cyanobacteria are typically known to proliferate in warm water environments such as tropical and temperate lakes and rivers, but they can also proliferate in cooler water environments under mesophilic and psychrophilic conditions (Seckback and Oren 2007). Cyanobacteria are also found in Antarctic habitats where they play a significant role in microbial ecosystem dynamics by providing fixed carbon via photosynthesis (Singh and Elster 2007). Cyanobacteria can grow in these extreme environments because they can adapt to survive freeze/thaw cycles and they can metabolize at near 0°C (Singh and Elster 2007).

The increase in water column stability associated with higher temperatures, less flow, and shallower water can also favor total cyanobacteria growth (Carey et al. 2012a; Wagner and Adrian 2009). In a study of 143 shallow lakes sampled along a latitudinal transect ranging from subarctic Europe to southern South America, Kosten et al. (2012) reported the percentage of cyanobacteria relative to total phytoplankton biovolume increased steeply with temperature in the lakes. The series of conditions most likely to result in cyanobacterial dominance begin with elevated winter–spring rainfall and runoff, followed by protracted periods of summer drought where temperatures, vertical stratification, and water residence times all increase simultaneously (Paerl and Otten 2013b).

Indirectly, warming can 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 and Andersen 1992; Søndergaard et al. 2003). Thus, increases in temperature can 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 can be explained not only through a temperature-related effect on the competitive advantage of cyanobacteria, but also by 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).

Cylindrospermopsis raciborskii was first identified in the tropics but has also been increasingly found in temperate regions since it was first found in North America in 1955 (Hong et al. 2006). *Cylindrospermopsis raciborskii* blooms are most likely to occur between the temperatures of 25 to 32°C but can sustain biomass at temperatures as low as 11°C (Antunes et al. 2015). 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 densities 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 levels of *C. raciborskii* only in the late summer, and these were associated with elevated bottom water temperatures and phosphorus concentrations.

3.1.1.3 Sunlight

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). The authors (Carey et al. and Falconer) found a greater proportion of the total phytoplankton biovolume attributable to cyanobacteria in lakes with high rates of light absorption. They could not establish cause and effect from their 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, primarily *Oscillatoriales* and other phytoplankton species (Scheffer et al. 1997; Smith 1986).

3.1.1.4 pH Levels

Total cyanobacterial blooms intensify and persist at pH levels between six and nine (Caraco and Miller 1998; WHO 2003a). Kosten et al. (2012) noted that pH affected cyanobacteria abundance in lakes along a latitudinal transect from Europe to southern South America. The percentage of cyanobacteria in the 143 shallow lakes sampled highly correlated with pH, increasing as the pH increased. Shapiro (1984) hypothesized that cyanobacteria have a competitive advantage over other phytoplankton species because they are efficient users of carbon dioxide in water. When dissolved carbon dioxide is high (low pH), conditions favor growth and replication of the green algal colonies over the blue-green cyanobacteria (Caraco and Miller 1998; Shapiro 1984). At alkaline pH levels, inorganic carbon is present as carbonate anion rather than as carbon dioxide, carbonic acid, or bicarbonate anion. This situation favors the growth of cyanobacteria because they can carry out photosynthesis when the levels of dissolved carbon dioxide are very low (high pH). The blue-green algae have a much higher photosynthetic demand for the dissolved carbon dioxide allowing them to out compete the green algae for the limited supply (Caraco and Miller 1998; Shapiro 1984). Thus, a higher water column pH can correlate with a higher proportion of cyanobacteria in an algal bloom.

The Caraco and Miller (1998) study suggests that pH and dissolved carbon dioxide, although chemically linked, are also independent factors in bloom dynamics because, even when dissolved carbon dioxide in

water is mechanically enriched, an alkaline pH still favors growth of the cyanobacteria over the green algae if nutrient inputs are constant.

3.2 Cyanotoxins

Much of the information and the studies summarized in this section for microcystins and cylindrospermopsin are described in detail in the EPA's HESDs and Drinking Water Health Advisories for microcystins and cylindrospermopsin (U.S. EPA 2015a, 2015b, 2015c, 2015d). The EPA's HESDs established the scientific basis for the EPA Drinking Water Health Advisories and also informed the EPA in developing these ambient water quality criteria (AWQC) or swimming advisories. This section summarizes the information that is provided in more detail in the EPA's HESDs. Additional information can be found in the EPA's HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c, 2015d).

3.2.1 Chemical and Physical Properties

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 for the different congeners (e.g., microcystin-LR is 995.17 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 the amino acids (Puddick et al. 2015).

The microcystin congeners are named based on their two variable amino acids (Carmichael et al. 1988). For example, microcystin-LR, the most common congener (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. In Figure 3-2, which shows the structure of microcystin-LR, leucine is in the X position and arginine is in the Y position. Table 3-1 lists the most common microcystin congeners, including the amino acids in the X and Y positions.

There are other variants of microcystins besides those that arise because of the two interchangeable amino acids on the microcystin ring. For example, demethylated congeners have been observed in Europe; Wejnerowski et al. (2018) identified demethylated forms of microcystin-RR and microcystin-LR in a toxigenic cyanobacterial bloom in Poland. Observations of demethylated microcystins suggest that more than 200 microcystin congeners are possible.

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

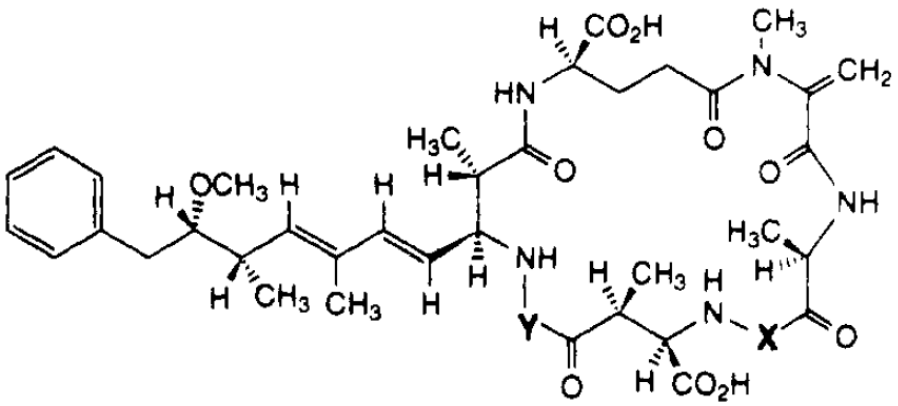


Table 3-1. Abbreviations for Selected Microcystins (Yuan et al. 1999)

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 result from tests using 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 (e.g., -LA, -LR, and -YR); the least toxic are those with hydrophilic amino acids, such as microcystin-RR (U.S. EPA 2015d; Ito et al. 2002; Rinehart et al. 1994; Vesterkvist and Meriluoto 2003; WHO 1999). Data on the -RR, -YR, and -LA congeners, however, are limited, and toxicity values cannot be derived for them. Therefore, values developed from data specific to microcystin-LR can represent other present 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 when the cell walls are broken down (cell lysis).

Cylindrospermopsin is a tricyclic alkaloid with the molecular formula of $C_{15}H_{21}N_5O_7S$ (Ohtani et al. 1992) and a molecular weight of 415.43 g/mole. It is a dipolar ion with localized positive and negative charges (Ohtani et al. 1992). The chemical structure of cylindrospermopsin is presented in Figure 3-3(a). Two naturally occurring congeners of cylindrospermopsin have been identified, 7-epi-cylindrospermopsin (the epimer of cylindrospermopsin) and 7-deoxycylindrospermopsin; see Figure 3-3(b) and (c) (de la Cruz et al. 2013; Norris et al. 1999). Recently, Wimmer et al. (2014) identified two new analogs, 7-deoxy-desulfo-cylindrospermopsin and 7-deoxy-desulfo-12-acetylcylindrospermopsin,

from the Thai strain of *Cylindrospermopsis raciborskii*. However, it is not clear if these are cylindrospermopsin congeners, precursors, or degradation products.

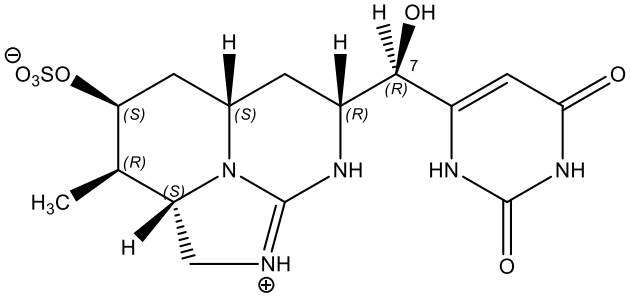
Table 3-2. Chemical and Physical Properites of Microcystin-LR

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	Not available (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 Octanol-Water Partition Coefficient (K_{ow})	2.16; -1.41 to 1.67 as pH decreases
Soil Organic Carbon-Water Partition Coefficient (K_{oc})	N/A
Solubility in Water	Highly*
Other Solvents	Ethanol and methanol

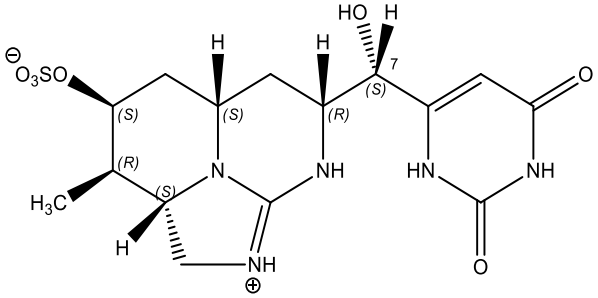
Sources: Chemical Book (2012); TOXLINE (2012); Ward and Codd (1999) and McCord et al. (2018) for log K_{ow}.
* Microcystin congeners vary in their relative solubility in water.

Figure 3-3. Structure of Cylindrospermopsin and Structurally Related Cylindrospermopsins (de la Cruz et al. 2013)

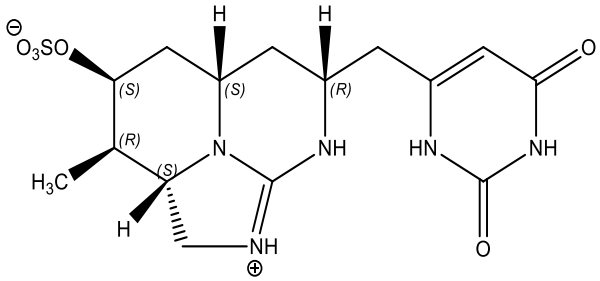
(a) Cylindrospermopsin



(b) 7-epi-cylindrospermopsin (the epimer of cylindrospermopsin)



(c) 7-deoxycylindrospermopsin



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). It is isolated for commercial use mostly from *Cylindrospermopsis raciborskii*. Some relevant physico-chemical properties of cylindrospermopsin could not be identified, and no physico-chemical properties were found for the structurally related cylindrospermopsins.

Table 3-3. Chemical and Physical Properties of Cylindrospermopsin

Property	Cylindrospermopsin
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 and methanol

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

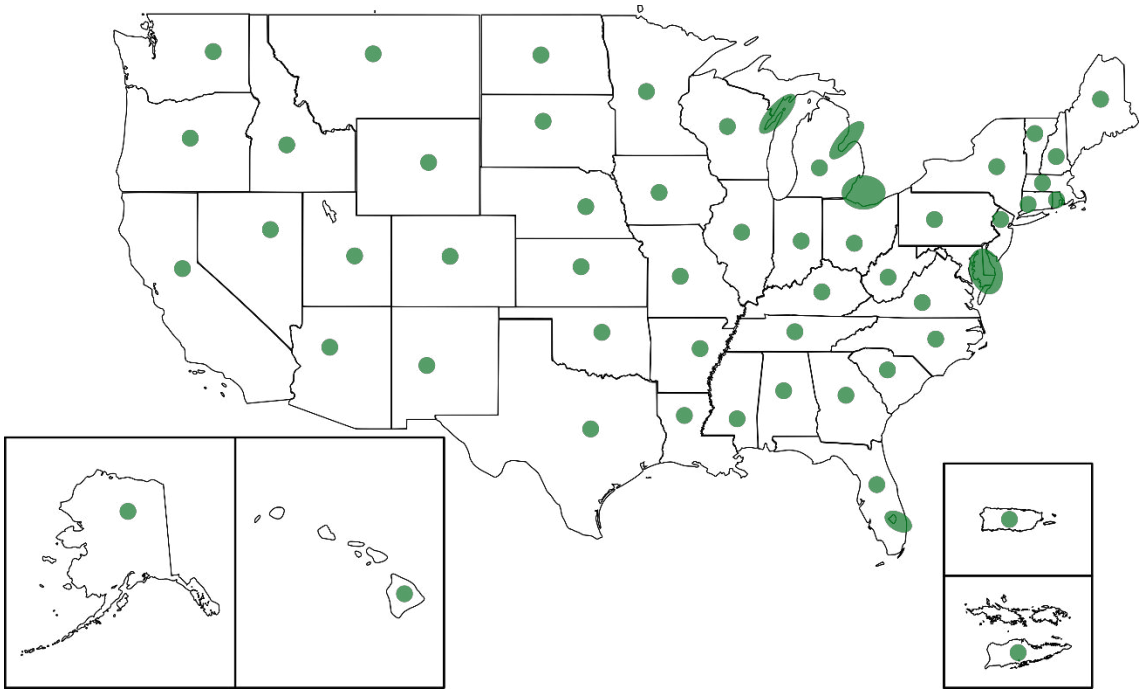
3.2.2 Sources and Occurrence in Surface Waters

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 3-4 (Richlen 2016; WHOI 2016). Figure 3-4 also identifies areas where more widespread HAB problems have occurred (e.g., parts of the Great Lakes).

Figure 3-5 shows the number of 2017 freshwater HAB recreational notices states publicly reported, organized by the EPA region between June 2 and August 1, 2017. To develop this regional summary map, the EPA researched and compiled publicly available reports posted on states' websites between these dates. During that time, states reported at least 281 notices for freshwater HABs with reported microcystin concentrations ranging from not detected (i.e., below the limit of detection) to 382 µg/L. These notices included cautions, warnings, public health advisories, and public health warnings due to

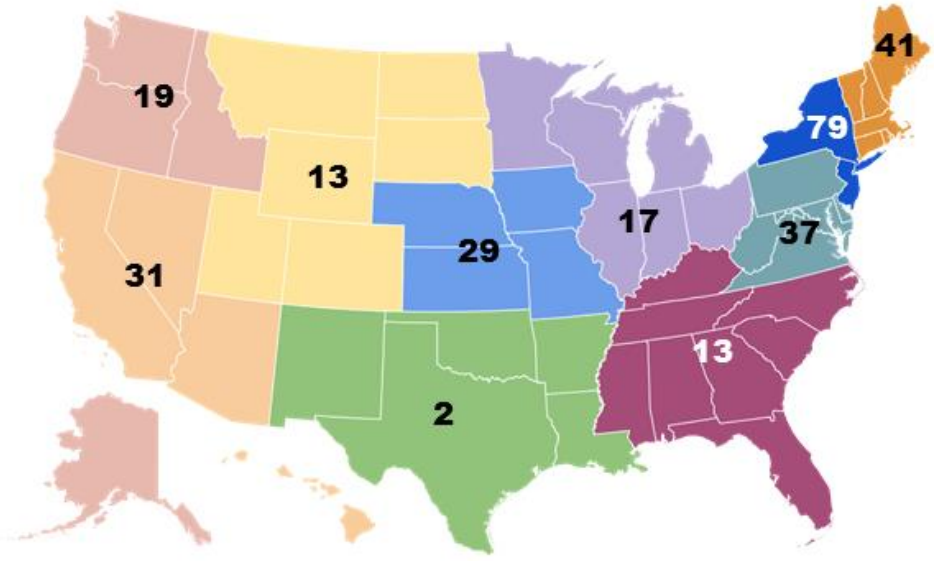
the presence of total cyanobacteria, cyanotoxins, or both. These notices can last for multiple days. The review was not exhaustive and might not reflect all the monitoring, beach, or general health advisories (e.g., some advisories at local or county-level may not be posted on the state website). Thus, the number of actual HAB notices during this time might be higher. In addition, many states have only recently begun to monitor HABs, so monitoring may be limited.

Figure 3-4. Generalized Distribution of Cyanobacterial HABs in the United States and Territories



[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 HAB is indicated with a single green dot. Larger green ovals mark areas where more widespread cyanobacterial HAB problems occurred.

Figure 3-5. State-reported HAB Notices by EPA Region, June 2 to August 1, 2017



3.2.2.1 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 and Testai 2008; Loftin et al. 2016b; U.S. EPA 2009). Dry-weight concentrations of microcystins in surface freshwater toxigenic 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 remainder of this section provides examples of microcystin concentrations reported in ambient waters in the United States.

The EPA (U.S. EPA 2009) reported on the 2007 National Lakes Assessment (NLA), a national probability-based survey of the nation's lakes, ponds, and reservoirs. The NLA 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 2007 NLA study surveyed 1,028 inland lakes and included measured microcystin concentrations, total cyanobacterial cell counts, and chlorophyll *a* concentrations. Microcystins were quantified using enzyme-linked immunosorbent assays (ELISA) with a detection limit of 0.1 µg/L (Loftin et al. 2016b). At each lake site, crews collected samples at a single station located at the deepest point in the lake and at ten stations around the lake perimeter. Due to the design of the survey, samples were taken at random and not necessarily where a bloom was occurring.

The 2007 NLA found that total cyanobacteria were detected in 98 percent of samples and were the dominant member of the phytoplankton community in 76 percent of samples (Loftin et al. 2016b; U.S. EPA 2009). Subsequent analysis indicated that potential microcystin-producing species occurred in 95 percent of samples (Loftin et al. 2016b). Microcystins were the most commonly detected class of cyanotoxins found in 32 percent of lakes in the contiguous United States (Loftin et al. 2016b; U.S. EPA 2009) and 39 percent of streams in the southeastern United States (Loftin et al. 2016a). Microcystins present in lakes ranged from the limit of detection (0.1 µg/L) to 225 µg/L with a mean concentration of 3.0 µg/L (detections only). Approximately 1.1 percent of lake samples exceeded 10 µg/L microcystins, and approximately 27 percent and 44 percent of lakes exceeded the WHO low-risk threshold for cyanobacterial abundance and chlorophyll *a*, respectively (Loftin et al. 2016b).

Lakes in states with microcystins levels > 10 µg/L reported in the 2007 NLA are shown in Table 3-4. The 2007 NLA data show two states (North Dakota and Nebraska) had nine percent of samples above 10 µg/L. Other states including Iowa, Texas, South Dakota, and Utah also had samples that exceeded 10 µg/L, but the frequency of detection was lower. Several of the 2007 NLA samples in North Dakota, Nebraska, and Ohio exceeded 20 µg/L (192, 225, and 78 µg/L, respectively).

In 2012, the EPA expanded on the 2007 NLA to include smaller water bodies in this statistically designed survey. Results represent the population of natural lakes, ponds, and reservoirs across the lower 48 states (not including the Great Lakes or the Great Salt Lake). To be included, in the survey lakes had to be larger than 2.47 acres (1 hectare), at least 3.3 feet (1 meter) deep, with a minimum quarter acre (0.1 hectare) of open water (U.S. EPA 2016). Data were collected from 1,038 lakes selected from a stratified random sample based on ecoregion, state, and surface area in the larger inference population (the set of 111,818 lakes). The NLA used thresholds established by the WHO to determine risk of exposure to cyanotoxins. Microcystins were detected in 39 percent of lakes monitored, but less than one percent exceeded the WHO estimates for microcystins at moderate or high risk of exposure. Less than one percent of lakes are in the most and moderately disturbed condition (i.e., have a high or moderate risk of exposure), and 99 percent are either least disturbed or show no detection of microcystins. Between 2007 and 2012, the percentage of lakes categorized as most disturbed for

microcystins did not change (U.S. EPA 2016), even though there was a significant increase in the detection of microcystins (+9.5 percent).

Table 3-4. States Surveyed as Part of the 2007 NLA with Water Body Microcystin 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.

The NLA used total cyanobacterial cell counts as an indicator of water quality impacts of microcystins; 15 percent of lakes were classified in the most disturbed condition, 23 percent were classified as moderately disturbed, and 61 percent were classified as least disturbed. Between 2007 and 2012, there was a statistically significant increase (+8.3 percent) in the number of lakes in the most disturbed category for cyanobacterial cell counts. Lakes that were considered most disturbed exceeded the WHO recreational levels of concern (20 µg of microcystins/L).

A survey conducted during the spring and summer of both 1999 and 2000 in more than 50 lakes in New Hampshire found measurable microcystin concentrations in all samples (Haney and Ikawa 2000). Microcystins were analyzed by ELISA and were found in all the lakes sampled with a mean concentration of 0.1 µg/L.

A survey conducted in Florida in 1999 found potential microcystin-producing genera in water samples, including, *Microcystis* (43.1 percent), *Anabaena* (28.7 percent), *Planktothrix* (13.8 percent), *Aphanizomenon* (7.2 percent), and *Coelosphaerium* (3.6 percent) (Burns 2008). Although *Planktothrix* and *Aphanizomenon* were found less frequently than were the other genera, at times they accounted for a significant portion of the cyanobacterial community present. Microcystins were the most commonly found toxins in Florida waters, occurring in all samples analyzed containing cyanotoxins (Burns 2008).

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.

A 2004 study of the Great Lakes found high levels of cyanotoxins 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 0.008 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 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). 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 (Hamel 2009, 2011, 2012).

In 2006, the U.S. Geological Survey (USGS) conducted a study of 23 lakes in the midwestern United States in which total 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. The researchers also found that cylindrospermopsin co-occurred with microcystins in nine percent of samples (Graham et al. 2010). Mixtures of all the microcystin congeners measured (-LA, -LF, -LR, -LW, -LY, -RR, and -YR) were common. Microcystin-LR and -RR were the dominant congeners detected with mean concentrations of 104 and 910 µg/L, respectively.

The Ohio EPA (2012) has been monitoring inland lakes since 2007 for cyanotoxins. Of the Ohio lakes sampled during the 2007 NLA, 36 percent had detectable levels of microcystins. In 2010, the Ohio EPA sampled Grand Lake St. Marys for cylindrospermopsin, microcystins, and other cyanotoxins. 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 microcystin levels exceeding 100 µg/L.

The USGS monitored Lake Houston in Texas from 2006 to 2008 and found microcystins in 16 percent of samples and at concentrations less than or equal to 0.2 µg/L (Beussink and Graham 2011). The USGS also 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). In 2011, the 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 microcystins were low in the majority of the tributaries with the exception of Milford Lake, which had higher total microcystin concentrations, some exceeding the Kansas 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 might 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 2008, the National Oceanic and Atmospheric Administration (NOAA) began monitoring for total cyanobacterial blooms in Lake Erie using high temporal resolution satellite imagery. Using the Great Lakes Coastal Forecast System, forecasts of bloom transport are created to estimate the trajectory of the bloom, which 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 six to eight 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 were separated into particulate (cell-bound) and dissolved (extracellular) phases

(Graham and 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. 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 microcystins (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).

A 2014 survey of southeastern U.S. streams detected microcystins in 39 percent of the samples (29 of 75 sites) (Loftin et al. 2016a). The stream sample concentrations ranged from the minimum reporting limit of 0.1 µg/L to 3.2 µg/L. In some cases, the source of the cyanobacteria in flowing water bodies was traced to an upstream water body such as a lake or reservoir.

From August to October 2015, a bloom identified as *Microcystis aeruginosa* occurred on the Ohio River (ORSANCO 2017). Patches of the bloom covered 636 miles of the river and peaked in late September. The Ohio River Valley Water Sanitation Commission (ORSANCO) collected over 150 river samples, which were analyzed for microcystins. Of the samples collected by ORSANCO, 15 (10 percent) were greater than 6 µg/L. The highest microcystin concentration was 1900 µg/L from a sample collected at river mile 468.8 (Cincinnati, Ohio). No toxins were detected in finished drinking water (tested by utilities and state agencies). Ohio, West Virginia, Kentucky, and Indiana issued recreation notices for the Ohio River as the bloom extended into their areas. Illinois issued a precautionary statement concerning recreation in the river due to concern that the bloom would reach their border. These recreation advisories were lifted after the bloom ended (ORSANCO 2017).

From July 14 to September 14, 2016, an extensive cyanobacterial bloom covering 100 square miles occurred in Utah Lake, Jordan River, and nearby canals. Microcystin-LR concentrations ranged from below the detection limit to 0.23 µg/L, and the highest total microcystin concentration reported was 176 µg/L (Utah Department of Environmental Quality 2016). Both maximum values were from samples collected at the surface near an accumulation of cyanobacteria. Cyanobacteria composition observed during the 2016 bloom varied in both time and space, but was primarily dominated by *Aphanizomenon* or *Dolichospermum*. Other taxa including *Geitlerinema*, *Pseudanabaena*, and *Phormidium* were also observed in significant densities in a few samples (Utah Department of Environmental Quality 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 contribute to the formation of massive cyanobacterial blooms (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). In July 2016, a 239-square mile cyanobacterial bloom in Lake Okeechobee was discharged and flowed through canals, rivers, and estuaries to the ocean. As a result of the microcystin levels in the river and at the coast, and the visible cyanobacterial scum in the lake and river, a state of emergency was declared in the counties of Martin, St. Lucie, Palm Beach, and Lee. From May 4 to August 4, 2016, 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). The microcystin concentrations in freshwater were reported in Lake Okeechobee (from not detected to 382.3 µg/L). Elevated levels were also reported in the St. Lucie River and the St. Lucie Canal (from not detected to 80.3 µg/L). Among the cyanobacteria species identified were *Microcystis aeruginosa*, *Scrippsiella trochoidea*, *Planktolyngbya limnetica*, *Dolichospermum circinalis*, and *Plectonema wollei* (Florida Department of Environmental Protection 2016).

3.2.2.2 Cylindrospermopsin

In general, fewer surface water occurrence data were available for cylindrospermopsin compared with microcystins. During blooms, testing for microcystins is much more common than is testing for cylindrospermopsin.

In a 1999 study, *Cylindrospermopsis* was detected in 40 percent of 167 water samples taken from 87 water bodies in Florida (Burns 2008). The actual cylindrospermopsin concentrations were not reported, but all samples containing the organism *Cylindrospermopsis* were positive for the toxin cylindrospermopsin.

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 and Clyde 2009).

The USGS detected cylindrospermopsin in nine 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 the genera *Aphanizomenon* or *Anabaena* and *Microcystis*.

The USGS analyzed the stored samples collected during the 2007 EPA NLA (U.S. EPA 2009) and detected cylindrospermopsin in four 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 (*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, and *Raphidiopsis*) occurred in 67 percent of samples (Loftin et al. 2016b). Cylindrospermopsins occurred most frequently in the midwestern and south-central United States and parts of Florida.

In Grand Lake in St. Marys, Ohio, cylindrospermopsin concentrations as high as 9 µg/L were reported in 2010 (Ohio EPA 2012).

3.2.3 Estuarine and Marine Waters

In Japan, the Isahaya Reservoir discharges water into Isahaya Bay. The reservoir experiences algal blooms seasonally, with species including nontoxic cyanobacteria as well a microcystin-producing *Microcystis aeruginosa* (Umehara et al. 2012). Water from the reservoir is discharged to the bay after rainfall events, even during periods of *Microcystis aeruginosa* blooms. Between November 2008 and November 2009, Umehara et al. (2012) estimated that 64.5 kilograms (kg) of microcystins were discharged to the bay, of which only 0.7 kg deposited on the floor. The authors speculated that because the majority of microcystins remain in the water, it is likely that they are washed out to other coastal areas with strong tides (Umehara et al. 2012).

In 2007, Miller et al. (2010) confirmed the presence of *Microcystis* and microcystins in Lake Pinto's downstream tributaries within 1 kilometer (km) of Monterey Bay in California after a large cyanobacterial bloom in the lake, and detected microcystins in nearshore marine waters following the

rainy season. The same researchers observed sea otters dying from consuming microcystin-contaminated clams, mussels, and oysters near ocean outflows of freshwater systems (Miller et al. 2010). A follow-up study was designed by Gibble and Kudela (2014) to identify the potential pathways leading to microcystin contamination in coastal ecosystems in and around Monterey Bay. They surveyed 21 sites at the land-sea interface in 2010–2011 followed by a survey of four watersheds in 2011–2013. In the first year of a three-year study, microcystins were detected in 15 of 21 freshwater, estuarine, and marine locations. In the two subsequent years, monitoring focused on four major watersheds that feed into Monterey Bay. The authors observed high microcystin concentrations 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 microcystins. Concentrations ranged from undetectable up to 20 ng/g resin, which translates to approximately 20 parts per billion (ppb) microcystins in the water column.

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. The use of assays targeting gene sequences for phycocyanin and microcystin synthase allowed the quantification of total and toxigenic *Microcystis*. Their results showed that large quantities of cyanobacterial cells could withstand passage through hydroelectric installations and transport over 300 km. Microcystin concentrations ranged from 165 µg/L in a reservoir upstream to 3.6 µg/L within the lower estuary less than 1 km from the Pacific Ocean (Otten et al. 2015).

The large cyanobacterial bloom in Lake Okeechobee, Florida, in 2016 (described above) flowed downstream and impacted estuarine and marine waters, resulting in beach closures along the Atlantic (Chaney 2016; Florida Department of Environmental Protection 2016). From May 4 to August 4, 2016, the Florida Department of Environmental Protection sampled freshwater, estuarine waters, and nearshore marine waters. The highest concentration reported (414.3 µg/L) was collected in Martin County at Bathtub Reef, a beach along the Atlantic Ocean. Sampling efforts in estuarine water, for example at a marina in the St. Lucie River, reported a concentration of 78 µg/L. The majority of marine waters sampled had low levels of microcystins (not detected or approximately 1 µg/L).

3.2.4 Other Sources of Microcystins and Cylindrospermopsin

Cyanotoxins have the potential to occur in drinking water, ground water, fish, shellfish, dietary supplements, air, soil, and sediments. These potential sources of cyanotoxins are discussed briefly in section 7.6. Exposure to these toxins in finished drinking water is also characterized in the Drinking Water Health Advisories (U.S. EPA 2015a, 2015b).

3.3 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.

3.3.1 Mobility

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). A study conducted by the USGS and the University of Central Florida determined that microcystin-LR 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-LR was due to biodegradation.

Cyanotoxins that are produced by cyanobacteria growing in freshwaters can enter estuarine and marine waters as waters containing the toxins flow downstream. Studies have demonstrated that toxigenic cyanobacteria can travel long distances in freshwater and can reach estuarine and marine waters from coastal lakes, reservoirs, and rivers (Preece et al. 2017).

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.

3.3.2 Persistence

3.3.2.1 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 two to three months in solution and up to six months in dry scum (Funari and 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 et al. (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 five 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 two weeks to longer than six weeks, depending on the concentration of pigment and the intensity of the light (Tsuji et al. 1994, 1995).

Several other factors, including 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, 1995), microcystin-LR was photodegraded with a half-life of about five days in the presence of 5 mg/L of extractable cyanobacterial pigment. Humic substances can act as photosensitizers and can increase the rate of microcystins breakdown in sunlight. Others have found that high concentrations of humic acids can slow the rate of microcystins 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 demonstrated that the wavelength of light can also affect degradation rates; complete microcystins degradation was observed within one hour when exposed to 254-nm light and within five 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 biodegradation 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 conducted by Jones et al. (1994) with microcystin-LR in different natural surface waters, microcystin-LR persisted for three days to three weeks; however, more than 95 percent loss occurred within three to four days. A study by Christoffersen et al. (2002) measured half-lives in the laboratory and in the field of approximately one day, driven largely by bacterial aerobic metabolism. These researchers found that approximately 90 percent of the initial amount of microcystins disappeared from the water phase within five days, irrespective of the starting concentration. Other researchers (Edwards et al. 2008) have reported half-lives of four 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 was observed with an optimal degradation rate at pH values 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, were not significantly degraded (Zastepa et al. 2014). During periods of high toxigenic cyanobacterial densities, the composition of other bacteria in the community may shift in response. In a study of the San Juan reservoir in Spain, Lezcano et al. (2017) found that several classes, orders, and families of known biodegrading bacteria, such as the *Spirobacillales* order, increased by more than a factor of 1.5 during the peak of a cyanobacterial bloom. The increase in relative abundance suggests that these biodegraders may play a role in microcystins degradation in the environment. Although microcystin-degrading bacteria might be present, initial degradation rates could be slow because the bacteria need time to begin using the toxins as carbon or energy sources (Hyenstrand et al. 2003). Microcystins can accumulate in the water column if these biodegrading bacteria are not present at the time of a toxic bloom (Schmidt et al. 2014). Cousins et al. (1996) demonstrated that microcystin experimentally added into reservoir water has a half-life of three to four days, whereas microcystin spiked into the same matrix but sterilized (so biodegrading bacteria are dead) had no significant change in the 12 days of the experiment. The authors concluded that biodegradation was the primary mechanism of microcystin reductions in the raw reservoir water.

Where rivers discharge to the ocean, freshwater cyanobacteria, cyanotoxins, or both can enter the marine environment (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. Microcystin concentrations in these experiments decreased in the range of 44 to 71 percent after one 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).

3.3.2.2 Cylindrospermopsin

Cylindrospermopsin is relatively stable in the dark and at temperatures from 4°C to 50°C for up to five weeks (ILS 2000). Cylindrospermopsin is also resistant to changes in pH and remains stable for up to eight 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 and Testai 2008).

Like microcystins, 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 two to three days (Chiswell et al. 1999).

Bacteria have been shown to decompose cylindrospermopsin in laboratory studies; the biodegradation is influenced by the cyanotoxin concentration, temperature, and pH. Mohamed and Alamri (2012) reported that *Bacillus* bacteria degraded cylindrospermopsin and that degradation occurred in six days at the highest toxin concentration (300 µg/L) and in seven or eight 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.

3.4 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 anion transporting 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 and 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, 1990b; Runnegar et al. 1995; Runnegar and 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 the EPA's Drinking Water Health Advisory and HESD for microcystins (U.S. EPA 2015a, 2015d).

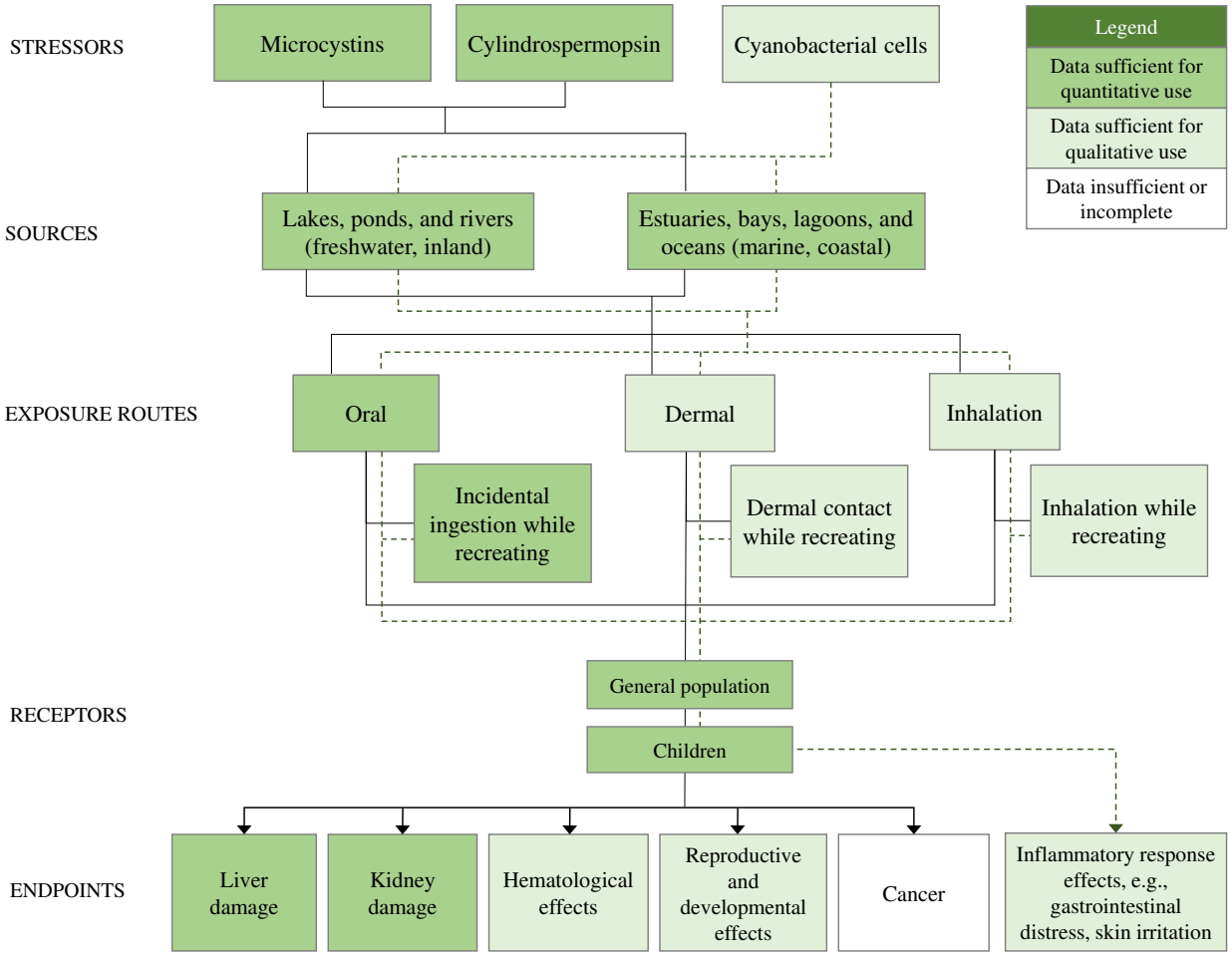
Limited toxicokinetic data for cylindrospermopsin are available and are derived from mice intraperitoneal studies and in vivo studies that do not necessarily reflect environmental exposure conditions (U.S. EPA 2015c; Pichardo et al. 2017). Cylindrospermopsin is absorbed from the GI tract (Humpage and 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 the EPA's Drinking Water Health Advisory and HESD for cylindrospermopsin (U.S. EPA 2015b, 2015c).

4.0 PROBLEM FORMULATION

4.1 Conceptual Model

This conceptual model provides useful information that characterizes and communicates the potential health risks related to exposure to microcystins and cylindrospermopsin in recreational waters. The model depicts the sources of the cyanotoxins in these waters, the recreational routes of exposure for sensitive biological receptors of concern, and the potential assessment endpoints (e.g., effects such as kidney and liver toxicity) (Figure 4-1).

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



4.1.1 Conceptual Model Diagram for Recreational Exposure

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 AWQC. Boxes that are shaded darker green indicate pathways that the EPA considered quantitatively in estimating the advisory level, whereas boxes shaded lighter green indicate data were sufficient for qualitative use and the white boxes did not have sufficient data for the EPA to

evaluate quantitatively or qualitatively. The solid lines are for the cyanotoxins and the dotted lines are for the cyanobacterial cells.

4.1.2 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. The EPA concluded that although statistically significant associations with adverse health effects occur across a wide range of cyanobacterial cell densities, criteria cannot be derived based on cyanobacterial cell density at this time. Effects related to cyanobacterial cells are discussed in section 7.5.1 and 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. This assessment focuses on cyanotoxins produced by these cyanobacteria in freshwater. These toxins have the potential to affect downstream waters, including coastal areas where surface water containing the toxins discharges into estuarine and marine waters.

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, or water skiing); and inhalation exposure to contaminated aerosols (while recreating). The route of exposure considered quantitatively in this assessment is the oral exposure to microcystins and cylindrospermopsin via incidental ingestion while swimming. Inhalation can occur from exposures from personal watercraft and boat spray. Dermal exposure can occur through recreational water contact; however, significant dermal absorption of microcystins and cylindrospermopsin is not expected due to the large size and charged nature of these molecules and the lack of dermal receptor sites capable of uptake (Butler et al. 2012; U.S. EPA 2004; U.S. EPA 2007). Sufficient data to quantify toxicity via the inhalation and dermal exposure routes were not available. The dermal and inhalation routes of exposure are discussed further in the Effects Characterization section (7.4).

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. 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. Therefore, the EPA has determined that childhood is the most vulnerable lifestage due to potential increased exposure while recreating when compared with adults. The EPA evaluates and discusses differences between lifestages in the Effects Characterization section (7.3).

While there are examples in the literature and reports of animal poisonings and death due to exposure of cyanotoxins, values protective of animals such as dogs and livestock are not generated in this document. However, section 7.8 discusses some animal-specific issues, including a summary of guidelines that 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 the EPA's HESD for microcystins (U.S. EPA 2015d).

Available cylindrospermopsin toxicity data are described in the EPA's HESD for cylindrospermopsin (U.S. EPA 2015c). For cylindrospermopsin, the EPA selected kidney effects as the endpoint on which to quantify the measure of effect. However, in both the critical study and the supporting studies there is evidence that cylindrospermopsin can also alter the shape of red blood cells.

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 self-reported health endpoints or combined symptom categories. Potential inflammatory health effects related to exposure to total cyanobacterial cells are described in the Effects Characterization section (7.5.1) and in Appendix D, both of which include a discussion of the uncertainties related to associations with cyanobacterial cells.

4.2 Analysis Plan

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

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

The EPA's HESD for microcystins and HESD for cylindrospermopsin (U.S. EPA 2015c, 2015d) provide the health effects basis for the development of the Drinking Water Health Advisories for microcystins and cylindrospermopsin (U.S. EPA 2015a, 2015b), including the basis for estimating the point of departure. To develop its HESDs for microcystins and cylindrospermopsin, the 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 EPA's HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c, 2015d).

The EPA's HESDs were subject to rigorous internal and external peer review before being 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. The EPA conducted supplemental literature searches to capture new references, including effects related to recreational exposure to cells. For detailed information on the search terms, see Appendix C.

4.2.1 Approach for Recreational AWQC and Swimming Advisory Derivation

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

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

Where:

RfD	=	reference dose (μg/kg body weight/day)
BW	=	mean body weight (kg)
IR	=	ingestion rate (L/day) (discussed in section 4.2.3.1)

4.2.1.1 Magnitude, Duration, and Frequency

Recreational criteria, like other 304(a) 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 water body that supports the designated use. Duration is the period over which the magnitude is calculated. Frequency of excursion describes the number of times the contaminant may be present above the magnitude over the specified period (duration). A criterion is derived such that the combination of magnitude, duration, and frequency protect the designated use (e.g., primary contact recreation).

4.2.2 Measures of Effect

The EPA's HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c, 2015d), provide the health effects basis for development of an oral toxicity value or the RfD, including the selection of the critical study and critical endpoints and application of uncertainty factors (UFs). In derivation of the recreational criteria and swimming advisory recommendations, the EPA uses these toxicity values as the measure of effect for oral exposure through incidental ingestion while recreating. The RfDs described in the EPA's HESDs are based on short-term and subchronic studies and therefore are 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 short-term exposure period.

4.2.3 Measures of Exposure

The EPA selected incidental ingestion during primary contact activities (such as swimming) in derivation of the recreational criteria and swimming advisories because data suggest that incidental ingestion can be considered the highest potential exposure pathway for cyanotoxins while recreating. Dorevitch et al. (2011) studied the volume of water ingested during a range of recreational activities in the Chicago Area Waterway System (CAWS) and at a public outdoor swimming pool. Study participants took part in one of the following activities on the CAWS: canoeing, fishing, kayaking, motor boating, or rowing. In the swimming pool, participants took part in canoeing, fishing, kayaking, swimming, or wading/splashing. 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. Therefore, the EPA determined that using a swimmer scenario for exposure as the basis for the criteria is protective of these other aquatic activities.

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 7.4.1 for a characterization of potential effects from inhalation exposure.

Dermal exposure happens during swimming; however, significant dermal absorption of the toxins 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, the EPA is not quantifying effects resulting from dermal exposure to cyanotoxins. See section 7.4.2 for a characterization of dermal exposure to these cyanotoxins.

Dermal exposure to cyanobacterial cells can also result in adverse health effects, such skin rashes, eye irritation, and ear irritation. Because adequate effects data are not available, the EPA is not quantifying effects resulting from exposure to cells at this time; effects are described qualitatively. Available epidemiological study results do not provide consistent associations between cell densities and the inflammatory health endpoints. See section 7.5.1 for a characterization of potential effects from recreational exposure to cyanobacterial cells.

All recreational exposure studies that included both children and adults found that age tended to influence incidental ingestion exposure while recreating. More specifically, children tend to ingest more water and spend more time in the water compared with adults (Dufour et al. 2017; Dufour et al. 2006; Schets et al. 2011; U.S. EPA 2011). Data supporting the selected exposure factors are described in the sections that follow.

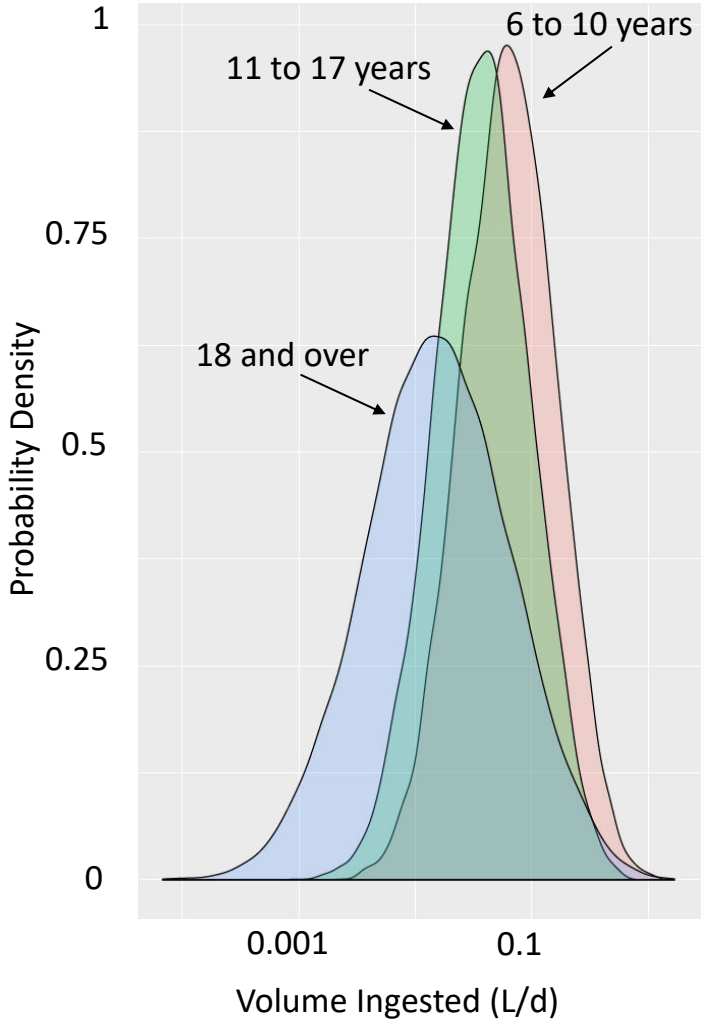
The measure of exposure is the 90th percentile of the daily incidental ingestion rate (volume of surface water incidentally ingested per day) and body weight (kg). Both body weight and incidental ingestion while recreating are parameters that vary with age. The EPA's 2000 Human Health Methodology (2000) outlines EPA's process for deriving AWQC and guides the development of these recreational criteria and swimming advisory recommendations.

4.2.3.1 Incidental Ingestion

To calculate the recreational incidental ingestion rate in units of volume per day, the EPA combined a distribution of incidental ingestion volumes (volume per event normalized to volume per hour) and a distribution of exposure durations (hours per day). The EPA uses the 90th percentile of the combined

distribution of ingestion rate and exposure duration to represent incidental ingestion per day, consistent with the EPA's Human Health Methodology (U.S. EPA 2000). Probability density plots of the combined distributions are shown in Figure 4-2. The ingestion data demonstrate that the mean ingestion rate for children six to 10 years is higher than for older children and adults. These data are discussed in the following sections.

Figure 4-2. Combined Distributions for Age Groups

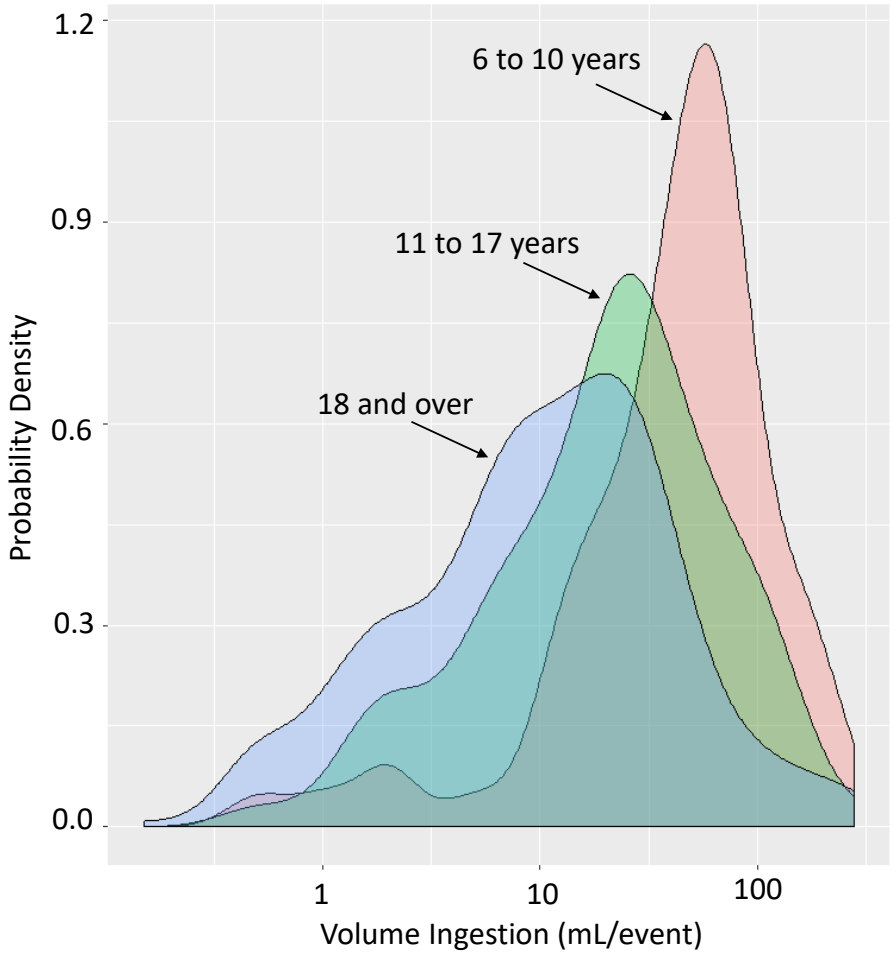


Ingestion Volume Studies

The EPA evaluated seven studies on ingestion and selected the dataset collected and analyzed by Dufour et al. (2017) for development of these AWQC or swimming advisory recommendations. This study used the same methodology as an earlier study (Dufour et al. 2006) but included 10 times more participants. Both studies used cyanuric acid as an indicator of amount of pool water ingested while swimming in an outdoor pool. 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 dataset collected by Dufour et al. (2017) included age information for each particpant ages six to 81 years, whereas the 2006 study classified individuals as over or under 18 years old. Both studies did not include children younger than six years old. The 2017

study recorded time spent in the water for each participant. The 2017 study results highlighted that younger children tested ingested more than older children or adults. The EPA selected the Dufour et al. (2017) dataset to calculate incidental ingestion volume because of the larger number of particpants, the inclusion of additional age groups, and recording of the duration exposure of each participant. The raw data collected and analyzed by Dufour et al. (2017) was provided by the study authors (U.S. EPA 2018a). The EPA adjusted (i.e., normalized) the volume ingested by each participant to one hour based on the length of time that participant reported being in the water. The summary statistics the EPA calculated using this dataset are shown in Appendix E (Table E-1). Figure 4-3 shows the raw data density plots for the Appendix E Dufour data separately grouped as age groups six to 10, 11 to 17, and 18 years and over. The density plots show the volume of incidental ingestion (mL) per recreational event on a log scale. To develop the distribution, each participant's volume ingested was adjusted to one hour based on the length of time that participant reported being in the water. Incidental ingestion was recorded for 66 individuals in the six- to 10-year category.

Figure 4-3. Incidential Ingestion for Age Groups Based on Appendix E Dufour Data



Appendix F describes seven studies that reported incidental ingestion while recreating, but only three others reported ingestion estimates for children (Dufour et al. 2006; Schets et al. 2011; Suppes et al. 2014). These other studies reported children's ingestion volumes similar to Dufour et al. (2017). Although these other studies corroborate the Dufour et al. (2017) findings, they were not selected for deriving the ingestion rate. Dufour et al. (2006) had fewer age groups (i.e., six to 17 and 18+ years), smaller sample size, and did not record time spent in water for each participant, making it a less robust

study than Dufour et al. (2017). Schets et al. (2011) collected data in the Netherlands, which may not be representative of the United States due to different behavioral trends in the resident population, including effects of temperature on recreating patterns. In addition, Schets et al. (2011) ingestion volumes are based on self-reported estimates; parents estimated volumes for children five and younger. Self- and parent-reported estimates are more uncertain than the methods used by Dufour et al. (2017). Suppes et al. (2014) used video and urine analysis to estimate ingestion volume. In Suppes et al. (2014) quantitative data were available for 35 participants, which is much lower than the sample size for Dufour et al. (2017). In addition, Suppes et al. (2014) only reported two age groups, children (five to 17 years) and adults (18+ years), which does not allow for the finer discernment of exposure patterns that is possible with the Appendix E and U.S. EPA (2018a) data.

Appendix F also describes the methodology used by the EPA's Office of Pesticide Programs (OPP) to calculate exposures to pool chemicals during swimming to support registration decisions. The Swimmers Exposure Assessment Model (SWIMODEL) (U.S. EPA 2003) uses incidental ingestion values for children that are twice the values used for noncompetitive adult swimmers. The model assumes an incidental ingestion rate of 0.050 L/hour for children ages seven to 10 years and 11 to 14 years while swimming noncompetitively. Incidental ingestion rates among adults while swimming competitively and noncompetitively are 0.0125 L/hour and 0.025 L/hour, respectively.

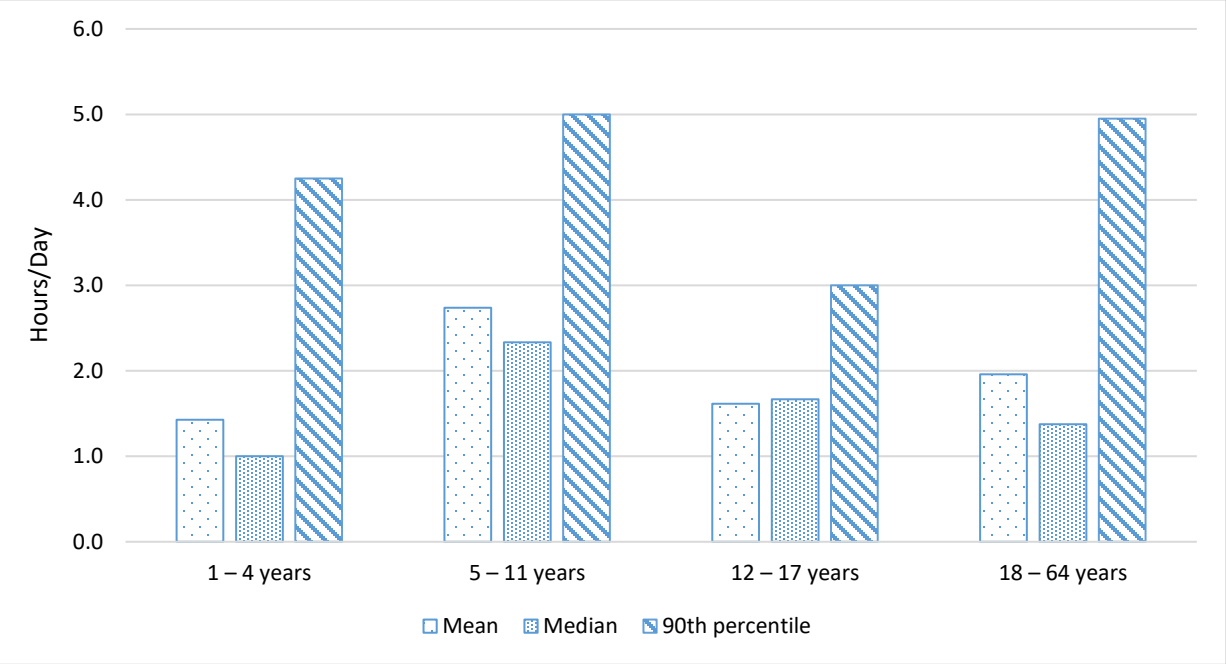
Duration of Recreational Exposure

Duration of recreational exposure quantifies the length of time people might be exposed to cyanotoxins during their primary contact recreational use. Duration 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 to derive the recommended cyanotoxin values.

The EPA selected recreational exposure data from the EFH (U.S. EPA 2011) for the development of these criteria/swimming advisories. The EPA's EFH (2011) lists time spent per 24 hours in an outdoor spa or pool for different age groups. The data are based on analysis of the National Human Activity Pattern Survey (U.S. EPA 1996). Figure 4-4 compares point estimates for the recreational duration data for different age groups and shows that recreators ages five to 11 years (n = 15) tend to spend more time in the water than other child age groups and adults. A duration was not provided for children younger than age one year.

The EPA investigated available exposure parameters for children younger than six years old, but they have large uncertainties given the lack of measured incidental ingestion data for this age group (see section 7.3.2). See section 7.2 (Recreational Exposure Duration) for further discussion of the available data for recreational exposure duration. The EPA used the distribution of exposure durations for children ages five to 11 years (n = 15; units are hour/day) as described below to calculate incidental ingestion per day.

Figure 4-4. Direct Contact Recreational Exposure Duration by Age Group, Based on Table 16-20 in U.S. EPA (2011)[a]



[a] This figure shows a comparison of point estimates. The EPA used the whole distribution for ages five to 11 years in deriving the AWQC and swimming advisory magnitudes.

Determination of Incidental Ingestion per Day

The incidental ingestion volume per day the EPA used to calculate the AWQC or swimming advisories is the product of the distribution of children's incidental ingestion rate for children ages six to 10 years (Appendix E; U.S. EPA 2018a) and the distribution of exposure durations for children ages five to 11 years (U.S. EPA 2011). The lifestage grouping for the duration data include children one year older and one year younger than the lifestage group for the incidental ingestion data.

The individual ingestion rate data points (adjusted to L/hour) were used to calculate a mean and standard deviation of the log-normal transformed dataset. This distribution was combined with the distribution of hours of recreation per day (ages five to 11 years) from the 2011 EFH (Table 16-20 *Time Spent (minutes/day) in Selected Outdoor Locations, Doers Only, At Home in the Outdoor Pool or Spa*). The mathematical relationship between the two variables and the daily incidental ingestion rate is shown in this equation:

$$\text{Ingestion Volume (L/hour)} \times \text{Recreation Duration (hour/day)} = \text{Daily Incidental Ingestion Rate (L/day)}$$

The EPA used probabilistic (Monte Carlo) simulation to develop the combined distribution of these variables as follows:

- Estimated statistical distributions for hourly ingestion rate and recreation duration for different age groups.
- Sampled randomly one value from each of these distributions.

- Multiplied the two sampled values.
- Repeated a large number of times (i.e., 100,000 times) to populate the distribution for daily ingestion rate (L/day) or the combined distribution.
- Reported results as summary statistics of the combined distribution.

The distribution shape that best fit the datasets was log-normal for both ingestion volume and exposure duration. Table 4-1 presents summary statistics for different age groups based on the combined distribution analysis. As per the EPA's 2000 Human Health Methodology (U.S. EPA 2000), the 90th percentile of exposure, represented by this combined distribution (0.21 L/day) was used as a point estimate for deriving the AWQC or swimming advisories. Details and the R code for this analysis are shown in Appendix E. Appendix E also includes the mean, median, and standard deviation for the distributions for ages six to 10, 11 to 17, and 18 years and older.

Table 4-1. Results of the Combined Distribution Analysis

Age Group	Summary Statistics for Ingestion Rate (L/day)		
	Median	Mean	90th Percentile
6 to 10 years	0.063	0.094	0.21
11 to 17 years	0.038	0.058	0.13
18+ years	0.015	0.04	0.10

4.2.3.2 Body Weight

Table 8-1 in the EPA's EFH (U.S. EPA 2011) reported body weight statistics based on the National Health and Nutrition Examination Survey, including for a range of age groups. The EPA selected children aged six to 10 years because it reflected the age group with higher ingestion volumes (Appendix E; U.S. EPA 2018a; U.S. EPA 2011) and exposure duration (U.S. EPA 2011).[4] As per the EPA's 2000 Human Health Methodology (U.S. EPA 2000), mean body weight (31.8 kg) was used for deriving the AWQC or swimming advisories. Section 7.3.2 provides a discussion of younger children's exposure factors.

4.2.4 Relative Source Contribution (RSC)

The RSC component of the AWQC calculation allows a percentage of the exposure to a contaminant to include other potential exposure sources. The RSC describes the portion of the RfD available for AWQC-related sources (U.S. EPA 2000); the remainder of the RfD is allocated to other sources of the contaminant. The EPA focused on recreational exposures to microcystins and cylindrospermopsin in ambient freshwaters. To derive recommendations protective of the recreational designated use, the EPA assumes all cyanotoxin exposure is from incidental ingestion of water while recreating; therefore, no RSC term is applied.

[4] The age group six to 10 years includes 10-year-old children. The EPA's *Exposure Factors Handbook* labels this age group as six to < 11 years.

5.0 EFFECTS ASSESSMENT

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

5.1 Hazard Identification

5.1.1 Noncancer Health Effects

5.1.1.1 Animal Toxicity Studies

Microcystins

The preponderance of animal toxicity data on the noncancer effects of microcystins is restricted to the microcystin-LR congener. Available data on the RR, YR, and LA congeners do not provide dose-response information sufficient for quantification. The EPA is using data on effects of microcystin-LR to represent other microcystin congeners (U.S. EPA 2015d). Observed effects in animals exposed orally or via intraperitoneal infusion to microcystin-LR include liver, reproductive, developmental, kidney, and GI effects (Chernoff et al. 2002; Falconer et al. 1998; Fawell et al. 1999; Fitzgeorge et al. 1994; Guzman and Solter 1999, 2002; Heinze 1999; Ito et al. 1997a, 1997b; Yoshida et al. 1997). Most oral and injection studies in laboratory animals have demonstrated that the liver is a primary target organ for microcystin toxicity. Liver effects, as well as kidney effects, have been reported in acute, short-term, and subchronic oral studies in laboratory animals exposed to microcystin-LR, in addition to reproductive effects following short-term and subchronic oral exposures. 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. For individual study details see the EPA's HESD for microcystins (U.S. EPA 2015d).

Available animal data on the acute oral toxicity of microcystin-LR provide evidence of hepatotoxicity. Liver effects described in the above studies are summarized in Table 5-1. A single oral dose of 500 μg microcystin-LR/kg resulted in diffuse hemorrhage in the liver of mice and rats; more pronounced liver damage occurred at higher doses (Ito et al. 1997a; Fawell et al. 1999; Yoshida et al. 1997). Studies that utilized parenteral administration of microcystin-LR show a steep dose-response with rapid onset of liver damage.

The findings in acute and subchronic studies support the liver as a target organ for microcystin-LR toxicity. The EPA identified a 28-day short-term study by Heinze (1999) as the critical study for derivation of an RfD. Male hybrid rats (10/group) were administered microcystin-LR in drinking water at doses of 0, 50, or 150 μg/kg body weight (Heinze 1999). Liver effects included increased liver weight, and slight to moderate liver necrosis lesions with or without hemorrhages at the low dose and with dose-related increases in necrotic severity. The necrosis was accompanied by changes in serum enzymes indicative of liver damage. All rats in each dose group had liver necrosis. Data were not collected prior to the end of the study so it is not known when during the 28-day study period these effects were manifested.

Table 5-1. Liver Effects in Animals Exposed to Microcystins in Selected Acute and Short-term Studies as Discusssed in the EPA's *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* (U.S. EPA 2015d)

Species	Exposure Route	Dosing Regimen	Micro-cystin Congener	Description of Liver Effects	Study
Female BALB/c mice (n = 7)	Gavage	Single dose of 0, 8,000, 10,000, or 12,500 µg/kg Examination at 24 hours after treatment	LR	Centrilobular hemorrhage, hepatocyte degeneration	Yoshida et al. (1997)
Male ICR mice aged (n = 29 age 32 weeks) and young (n = 12 age 5 weeks)	Gavage	Single dose of 500 µg/kg Animals sacrificed at 2, 5, and 19 hours after treatment	LR	Bleeding and disappearance of hepatocytes in the whole liver or in centrilobular region, friable tissue, necrosis, or eosinophilic changes in the centrilobular region	Ito et al. (1997a)
CR1:CD-1(ICR)BR(VAF plus) mice and CR1:CD(SD)BR(VAF plus) rats (5 males and 5 females per group)	Gavage	Single dose of 500, 1,500, or 5,000 µg/kg (no control) Animals sacrificed at day 14 post treatment	LR	Darkly discolored and distended livers; moderate or marked centrilobular hemorrhage of liver; diffuse hemorrhage in the liver	Fawell et al. (1999)
Male ICR mice (n = 5 per group)	Gavage	Repeated doses of 0, 4.6, 23, 46, 93, or 186 µg/kg/day for 7 days Animals sacrificed at day 7	RR	Dose-dependent increase in apoptosis	Huang et al. (2011)
Male hybrid rats (F1 generation of female WELS/Fohm × male BDIX) (10 per group)	Drinking water	Repeated doses of 0, 50, or 150 µg/kg/day for 28 days	LR	Hepatocyte degeneration, hemorrhage, and necrosis; increase in periodic acid-Schiff-positive substances (indicating cell damage), Kupffer cell activation	Heinze (1999)
Male Sprague-Dawley rats (3 per group)	Intraperitoneal infusion	Repeated doses of 0, 16, 32, or 48 µg/kg/day for 28 days	LR	Fibrous tissue, cell death, necrosis, lipid vacuoles, Kupffer cell activation (+2 and +3 severity rating)	Guzman and Solter (1999)

The liver effects in the Heinze (1999) study were supported by additional data from a study by Guzman and Solter (1999). Rats exposed via intraperitoneal infusion displayed histological evidence of liver

damage (i.e., inflammation, fibrous tissue, necrosis, and apoptosis). The study authors identified a no-observed-adverse-effect-level (NOAEL) of 16 μg/kg/day and a lowest-observed-adverse-effect-level (LOAEL) of 32 μg/kg/day. Microcystin-LR was delivered directly to the livers of the animals in the study by implanted osmotic pumps and this may account for the liver effects observed at lower doses compared to Heinze (1999). Guzman and Solter (1999) only included three rats per group exposed to doses of 0, 16, 32, or 48 μg/kg/day of microcystin for 28 days, which is a limitation of the study design. Although adverse liver effects were observed, the limited numbers of animals per dose group (n = 3) and the exposure route, which bypassed intestinal barriers to absorption, resulted in greater uncertainty than Heinze (1999). Thus, Guzman and Solter (1999) was not used to derive the RfD.

Some studies observed other kinds of effects following short-term or subchronic oral or intraperitoneal exposures. These studies, including limitations, are discussed in the EPA's HESD for microcystins (U.S. EPA 2015d). Potential effects included reproductive toxicity in males (Chen et al. 2011), maternal mortality (Fawell et al. 1999; Chernoff et al. 2002), and fetal body weight changes (i.e., at 2,000 μg/kg, administered orally during gestational days six to 15, at which significant maternal mortality was observed) (Fawell et al. 1999). Chernoff et al. (2002) did not report adverse effects on fetal or pup weights in two separate intraperitoneal studies.

Cylindrospermopsin

The available acute, short-term, and subchronic studies for cylindrospermopsin (Bazin et al. 2012; Humpage and Falconer 2002; 2003; Reisner et al. 2004; Terao et al. 1994; Shaw et al. 2001) support the liver and kidneys as the primary targets for cylindrospermopsin toxicity (summarized in Table 5-2), with effects on red blood cells also evident. These effects were observed in mice given single or repeated doses of purified cylindrospermopsin via oral administration or intraperitoneal injection (Bazin et al. 2012; Humpage and Falconer 2002, 2003; Reisner et al. 2004; Terao et al. 1994). The EPA did not find health effects information for other cylindrospermopsin congeners or analogs.

No oral reproductive or developmental studies are available for cylindrospermopsin. Developmental toxicity studies following intraperitoneal 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 individual study details, see the EPA's HESD for cylindrospermopsin (U.S. EPA 2015c).

The RfD for cylindrospermopsin was derived from the 11-week critical study by Humpage and Falconer (2002, 2003). This study was an 11-week study in mice, and the critical effect identified was kidney toxicity. The short-term studies available for cylindrospermopsin (Shaw et al. 2001; Reisner et al. 2004), were also evaluated and are considered supportive of the critical study; however, the EPA concluded that they were not suitable for quantification based on limitations including the use of extract, lack of adequate numbers of animals, monitored endpoints, the limited number of doses tested and endpoints monitored.

Humpage and Falconer (2002, 2003) identified a NOAEL of 30 μg/kg/day and a LOAEL of 60 μg/kg/day for increases in relative kidney weight in mice treated with purified cylindrospermopsin by gavage for 11 weeks. There were indications of reduced renal function effects, decreased urinary protein, and red blood cell effects (including increased bilirubin, spleen weight and polychromasia, indicative of hemolysis) at doses above the LOAEL. Although effects on kidney weight and urine protein levels were observed in male mice, the biological relevance of the latter effect and whether it would also occur in female mice needs further investigation. Mice are known to excrete a group of

highly polymorphic, low-molecular-weight urinary proteins that play important roles in social recognition and mate assessment. The relevance of the urinary protein findings in mice to humans is unknown. Humpage and Falconer (2002, 2003) found signs indicative of hemolysis (e.g., increased bilirubin, spleen weight and polychromasia), however these changes were not statistically significant.

Results from Reisner et al. (2004) corroborate Humpage and Falconer (2002, 2003) with comparable effects observed in mice during a three-week study. The kidney and red blood cell effects observed by Reisner et al. (2004) occurred at a LOAEL of 66 μg/kg/day in drinking water. The study authors demonstrated significant increases in hematocrit, acanthocytes (abnormal red blood cells), and liver and testes weights in exposed animals and a duration-related nonsignificant increase in kidney weight. The red blood cell effects were seen as early as the end of the first week of dosing and were present in each of the three weekly blood samples collected. Sukenik et al. (2006) observed similar effects on red blood cells (increases in hematocrit from week 16 to 32 accompanied by increased numbers of acanthocytes up to week 42) in male and female mice exposed to gradually increasing concentrations of cylindrospermopsin (i.e., from 100 to 550 μg/L) in drinking water for 42 weeks. Mice were given cylindrospermopsin in the form of spent medium on which cultures of *Aphanizomenon ovalisporum* had been grown; other medium components were not characterized. The authors proposed a LOAEL of 20 μg/kg/day (equivalent to 200 μg/L) for male and female mice based on changes in hematocrit at 16 weeks (Sukenik et al. 2006). This study was not selected as a critical study because this study used a single dose; however, the kidney and red blood cell effects at that dose after three weeks were comparable to the effects seen in the Humpage and Falconer (2002, 2003) study at a slightly lower 60 mg/kg/day dose after 11 weeks.

The short-term study by Shaw et al. (2001) was also considered in the development of the RfD for cylindrospermopsin. Shaw et al. (2001) reported liver effects (fatty infiltration) in mice given 50 μg/kg purified cylindrospermopsin by gavage for 14 days; this dose is lower than the NOAEL identified in the key study by Humpage and Falconer (2002, 2003). However, the EPA concluded that the Shaw et al. (2001) study was not suitable for quantification based on the limited number of doses tested.

A 90-day oral toxicity study by Chernoff et al. (2018) demonstrated signs of hepatic and renal injury in mice at all dose levels (0, 75, 150, and 300 μg/kg/day). Liver toxicity effects were noted by elevated absolute and relative liver weights, increases in serum alanine aminotransferase activity, reduced serum blood urea nitrogen and cholesterol levels, and increased incidence of hepatocellular hypertrophy and cord disruption. Renal toxicity effects were demonstrated in elevated absolute and relative kidney weights and renal cellular hypertrophy, tubule dilation, and cortical tubule lesions. Males showed more susceptibility to toxic effects; liver and kidney/body weight ratios, reduced cholesterol levels, cellular signs of inflammation, and degree and extent of renal histopathological damage were all observed to be more prominent in males. A NOAEL was not determined for any dose level based on significant liver and kidney effects exhibited in the 75 μg/kg group. The LOAEL of 75 μg/kg observed by Chernoff et al. (2018) is higher than the Humpage and Falconer (2002, 2003) NOAEL of 30 μg/kg.

Table 5-2. Kidney and Liver Effects in Animals Exposed to Cylindrospermopsin (Purified) in Acute and Key Short-term Studies in the *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA 2015c)

Species	Exposure Route	Dosing Regimen	Description of Kidney and Liver Effects	Study
Male Swiss albino mice (10 mice per group, except the highest dose group, which included 6 mice)	Gavage	Repeated doses of 0, 30, 60, 120, or 240 µg/kg/day for 11 weeks	Kidney: dose-related increases in relative kidney weight, proximal renal tubular damage, decreased urinary protein Liver: necrosis, inflammatory foci, and bile duct changes	Humpage and Falconer (2002, 2003)
CD-1 (Swiss-Webster) mice (18 to 20 per group)	Gavage	Repeated doses of 0, 75, 150, or 300 µg/kg/day for 90 days	Kidney: elevated absolute and relative kidney weights, renal cellular hypertrophy, tubule dilation, cortical tubule lesions Liver: elevated absolute and relative liver weights, increases in serum alanine aminotransferase activity, reduced serum blood urea nitrogen and cholesterol levels, increased incidence of hepatocellular hypertrophy and cord disruption	Chernoff et al. (2018)
Male Swiss mice (3 per group)	Gavage	Single dose of 1,000, 2,000, or 4,000 µg/kg Examination at 24 hours after treatment	Liver: dark red liver, apoptosis in the liver and the kidneys	Bazin et al. (2012)
Male ICR mice (n = 24, single group)	Intraperitoneal injection	Single dose of 200 µg/kg Three animals sacrificed at 8 time points, 16–100 hours after treatment	Kidney: proliferation of the endoplasmic reticulum and fat droplet accumulation in cells along the brush borders of the tubules plus limited single cell necrosis Liver: necrosis in the centrilobular region	Terao et al. (1994)
Male ICR mice (4 per group)	Drinking water	Repeated doses of 0 or 0.6 mg/L (estimated at 66 µg/kg/day) for 3 weeks	Kidney: duration-related nonsignificant increase in kidney weight Liver: increases in relative weight	Reisner et al. (2004)
Quackenbush mice (4 per group)	Intraperitoneal injection	Single dose of 200 µg/kg	Liver: fatty infiltration and cell necrosis	Shaw et al. (2001)

Species	Exposure Route	Dosing Regimen	Description of Kidney and Liver Effects	Study
Quackenbush mice (4 per group)	Gavage or intraperitoneal injection	0 to 300 μg/kg/day (oral) or 0 to 25 μg/kg/day (intraperitoneal injection) for 14 days	Liver: fatty infiltration (oral), foamy hepatocellular cytoplasm (intraperitoneal injection)	Shaw et al. (2001)

The Humpage and Falconer (2002, 2003) study was determined to be the most appropriate for the quantitative assessment because the LOAEL at 11 weeks would be protective for the effects seen at three weeks in the shorter duration study. For these reasons, this RfD was deemed suitable for development of the short-term drinking water health advisory and for use in recreational exposure scenarios. The EPA's HESD and Health Advisory documents for cylindrospermopsin describe the selection of the critical study and effect in detail and provide the rationale for applicability of the longer-term duration study (U.S. EPA 2015c).

5.1.1.2 Human Studies

Microcystins

The EPA identified the available epidemiological, outbreak, and case study reports on adverse health effects from oral exposures to microcystins. Limited human studies examining microcystin effects on humans exposed via drinking water are available, and no dose response data from oral exposure to microcystins in ambient water were identified. 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). The EPA identified four epidemiological studies, three case reports, and two outbreak summaries that evaluated human health effects associated with recreational exposures to cyanobacteria and microcystins. This human health effects information is summarized in the paragraphs that follow.

Backer et al. (2008) characterized microcystin concentrations in blood and reported symptoms in people recreating in a lake with a *Microcystis 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 were low and ranged from 2 to 5 μg/L. Recreational users of the lake at the time of the bloom had no detectable microcystins 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 (n = 104) and included a limited number of exposure days in the analysis (three days). The study demonstrated that people recreating on or in a water body can be exposed to aerosolized microcystins. However, given the limited number of participants and exposure days, and the low levels of microcystins present in the water and as aerosols, there were no reported increases in self-reported symptoms following recreational exposures. Other symptoms consistent with microcystin intoxication (e.g., liver toxicity) were not included in the study.

Backer et al. (2010) applied the same experimental approach at three lakes in California. Two of the lakes experienced blooms producing much higher microcystin concentrations compared with the lakes studied in the Backer et al. (2008) study, and the third lake did not contain a toxin-producing bloom. Eighty-one people, aged 12 and older, participated in the study and engaged in waterskiing, using personal watercraft, swimming, or wading. Total microcystins present in the lake containing toxic blooms ranged from < 10 µg/L to > 500 µg/L. Measured microcystin concentrations from personal air samples ranged from the limit of detection (0.1 ng/m^3) to 2.89 ng/m^3; the mean air concentration was 0.4 ng/m^3. Similarly, nasal swabs ranged from below the limit of detection to 5 ng, and all blood samples were below the limit of detection. Recreators had a significantly higher amount of microcystins present in nasal swabs after exposure. 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. Other symptoms consistent with microcystin intoxication (e.g., liver toxicity) were not included in the study. Adenoviruses or enteroviruses were not detected at the study locations. The authors concluded that it is possible for microcystins to become aerosolized, which in turn represents a potential route of exposure to recreators. They recommended additional research studying larger populations and sensitive subgroups.

Lévesque et al. (2014) conducted a prospective study of residents living in proximity to three lakes in Canada affected by cyanobacteria and microcystins 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 symptoms (e.g., ear pain, muscle pain). 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. The authors reported a dose-effect relationship (p-trend = 0.001) between total cyanobacterial cell counts and severe GI illness with a significant increase in reported symptoms starting at 20,000 cells/mL and above. The study reported a relative risk value of 3.28 (95 percent confidence interval (CI): 1.69–6.37) for the more severe GI symptom index (i.e., GI2, defined as diarrhea or vomiting or (nausea and fever) or (abdominal cramps and fever)) for exposures by full or limited contact to concentrations higher than 100,000 cells/mL (Lévesque et al. 2014). Adjusted relative risks of GI illness were significantly high for limited contact, but no relationship was found between GI symptoms and full contact. The authors explained that study participants avoided full contact with lake waters when high densities of cyanobacteria were visible, but continued to have limited contact. No significant fecal contamination measured by *Escherichia coli* (*E. coli*) was observed with geometric means in the lakes ranging from 8 to 145 colony forming units (CFU)/100 mL.[5] No associations were observed between any symptoms and recreational exposures to microcystins. Overall, the microcystin concentrations were low during the study, and the reported lower bound of the upper tertile was 0.2456 µg/L. The maximum microcystin concentrations for which recreational-related GI symptoms were reported was 7.65 g/L; however, microcystins occurred at much higher concentrations (e.g., maximum reported microcystin concentrations of 108 µg/L and 773 µg/L at two of the study locations), but there was no significant trend of increasing illness symptoms with elevated toxin concentrations. The study did not characterize the primary endpoint of concern for exposure to microcystins (i.e., liver toxicity) and did not conduct the necessary medical testing to determine liver function impairment.

Lévesque et al. (2016) provided additional analysis of the prospective study reported previously. Because GI illness was significantly associated with increasing cyanobacterial cell densities and GI

[5] Current Canadian recreational water guidelines for *E. coli*: geometric mean ≤ 200 *E. coli*/100 mL and single-sample maximum ≤ 400 *E. coli*/100 mL (Health Canada 2012).

symptoms can be related to cellular constituents, also termed *endotoxins* in the literature, the authors characterized the relationship between endotoxin exposure and illness in the study participants. Endotoxins include cell wall-associated lipopolysaccharides present in cyanobacteria and Gram negative bacteria. Frozen filters collected during the study were analyzed for endotoxins. The authors found a weak correlation between endotoxin levels and cyanobacteria cell density and reported a significant trend of increasing GI illness with increasing endotoxin concentrations. They also suggest that endotoxin concentrations could be a surrogate for another stressor. They cite other researchers that have suggested the endotoxins could be contributed by other members of the microbial community or the reported symptoms could be related to another stressor (Berg et al. 2008; Blahova et al. 2013; Rapala et al. 2002; Stewart 2006d).

In a recent case report by Vidal et al. (2017), a 20-month-old child and three adults reported GI symptoms several hours after engaging in bathing and other recreational activities at beaches in Montevideo, Uruguay, during January 2015. At that time, a cyanobacterial bloom of mainly *Microcystis* occurred in the River de la Plata. While the GI symptoms in the adults (i.e., diarrhea) rapidly resolved, the child's symptoms (i.e., diarrhea and vomiting) persisted. The child developed fatigue and jaundice, and five days after the exposure, she was admitted to hospital. Tests showed significant increases in bilirubin and serum liver enzymes, and a diagnosis of acute liver failure was given. The child was recommended for, and received, a liver transplant. The city government's beach monitoring program from April 2014 to March 2015 reported mean and maximum microcystin concentrations of 2.9 µg/L of 56 µg/L, respectively. These levels were reported in water samples from the beaches the family used with cyanobacteria presence but without cyanobacterial foam. Mean and maximum microcystin concentrations of 2,900 µg/L and 8,200 µg/L, respectively, were reported in water samples with cyanobacterial foam. The monitoring program also reported geometric means of fecal coliform values below the limit of 1,000 CFU/100 mL. After the child received a liver transplant, histological analysis of the explanted liver revealed liver damage characterized by hemorrhagic necrosis, intracytoplasmic cholestasis, large and multinucleated hepatocytes, proliferation, and nodular regeneration. The pathological findings and detection of microcystin-LR in the liver (2.4 ng microcystin-LR/g and 75.4 ng (D-Leu1) microcystin-LR/g liver) led to a diagnosis of acute liver failure related to exposure to microcystin-LR and cyanobacteria.

In another case report, acute intoxication with microcystin-producing cyanobacterial blooms in recreational water was reported in Argentina in 2007 (Giannuzzi et al. 2011). A male Jet Skier was exposed to 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. The subject was immersed for two hours as a result of an accident that required him to swim to the shoreline towing the Jet Ski. Four hours later the subject reported experiencing nausea and abdominal pain. Three days later the subject sought medical assistance because of respiratory distress requiring his hospitalization. One week after the exposure, the patient developed a hepatotoxicosis with a significant increase of serum alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase. With treatment, the patient recovered within 20 days.

An outbreak among army recruits undergoing canoe exercises who had consumed reservoir water containing a bloom of *Microcystis aeruginosa* reported symptoms of headache, sore throat, vomiting and nausea, stomach pain, dry cough, diarrhea, blistering around the mouth, and pneumonia (Turner et al. 1990). Microcystins, including microcystin-LR, were present in bloom samples. However, high levels of *E. coli* were also found in reservoir water after two weeks. The authors suggested that exposure

to microcystins may have had a role in some of the clinical symptoms; however, this case report information is insufficient to establish cause and effect.

Dziuban et al. (2006) and Hilborn et al. (2014) reported 10 outbreaks associated with recreational exposure to cyanobacteria in which microcystins were detected. Hilborn et al. (2014) reported that eight of these investigations evaluated the presence of cyanotoxins; eight detected microcystins; and two detected cylindrospermopsin. In four of the outbreaks, microcystin concentrations ranged from 0.2 μg/L to > 2,000 μg/L. Four outbreaks had microcystin concentrations > 20 μg/L. Cylindrospermopsin and anatoxin-a also were detected in three of the outbreaks. In one outbreak, 20.8 μg/L microcystins was measured, and other cyanotoxins were either not detected or measured. The nine persons reporting illness for this outbreak had symptoms that included abdominal cramps (3 people), diarrhea (3), nausea (3) vomiting (2), fever (2), headache (2), rash (8), eye irritation (1), ear ache (1), neurologic symptoms (2), tingling (2), confusion (1), and respiratory symptoms (1) (Hilborn et al. 2014). Dziuban et al. (2006) reported on two 2004 cyanobacteria-associated outbreaks in which 22 cases of illness were associated with elevated levels of microcystins in Nebraska lakes. The predominant illnesses in both outbreaks included dermatitis and gastroenteritis, and individuals who sought medical care showed a combination of rashes, diarrhea, cramps, nausea, vomiting, and fevers. Walker et al. (2008) also reported about a Nebraska outbreak. Levels of total microcystins at the east swimming beach of Pawnee Lake exceeded 15 ppb on July 12, 2004, and a health alert was issued. However, heavy public use of Pawnee Lake occurred that weekend and more than 50 calls were received from the public, complaining about symptoms such as skin rashes, lesions, blisters, vomiting, headaches, and diarrhea after swimming or water skiing in Pawnee Lake (Walker et al. 2008). The outbreak reports data are not sufficient to establish cause and effects for microcystins because of weaknesses in the nature of the data reported and the many potential confounding variables. 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 these types of outbreaks occur during the warmer months. Hilborn et al. (2014) noted that HAB-associated illness from recreational exposure might be underreported due to multiple possible exposure routes and the non-specific nature of potential health effects.

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 and Bartram 1999; Hilborn et al. 2014; Huisman et al. 2005; Yoo et al. 1995).

Information on the human health effects of microcystins based on epidemiological studies related to drinking water exposures to microcystins are discussed in detail in the EPA's HESD for microcystins (U.S. EPA 2015d). These studies are summarized in the paragraphs that follow.

An epidemiology study done in Australia compared the hepatic enzyme levels from patients served by a public water supply contaminated with a *Microcystis aeruginosa* bloom with enzyme levels from patients living in areas served by water supplies uncontaminated by cyanobacteria (Falconer et al. 1983). Although the authors observed significant variability in enzyme levels between the two groups, the findings were attributed by the authors to the imprecise method of study participant selection and confounding factors such as alcoholism and chronic kidney disease among some of the participants.

A cross-sectional study conducted in China assessed the relationship between the consumption of drinking water and aquatic food (carp and duck) contaminated with microcystins and liver damage in children (Li et al. 2011b). The authors found that mean serum levels of microcystins ranged from below detection to 1.3 μg microcystin-LR equivalents/L. According to the authors, hepatitis B infection was a greater risk for liver damage among these children than the microcystins exposure.

An outbreak of acute liver failure occurred in a dialysis clinic in 1996 in Caruaru, Brazil, where dialysis water was contaminated with microcystins, and possibly cylindrospermopsin. Of the 130 patients who received their routine hemodialysis treatment (intravenously) at that time, 116 reported symptoms of headache, eye pain, blurred vision, nausea, and vomiting. Subsequently, 100 of the affected patients developed acute liver failure and, of these, 76 died (Carmichael et al. 2001; Jochimsen et al. 1998). Analyses of blood, sera, and liver samples from the patients revealed microcystins.

In another contamination event at a dialysis center in Rio de Janeiro, Brazil, in 2001, 44 dialysis patients were potentially exposed to microcystin concentrations of 0.32 μg/L, detected in the activated carbon filter used in an intermediate step for treating drinking water to prepare dialysate (Soares et al. 2005). Concentrations of 0.4 μg/L microcystin-LR were detected in the drinking water. Serum samples were collected from 13 dialysis patients 31 to 38 days after the detections in water samples, and patients were monitored for eight weeks. Concentrations of microcystin-LR in the serum ranged from 0.46 to 0.96 ng/mL. Although the biochemical outcomes varied among the patients, markers of hepatic cellular injury and of chlolestasis (elevations of AST, ALT bilirubin, ALP, and GGT) in serum during weeks one to eight after treatment frequently exceeded normal values (Hilborn et al. 2013). Because microcystin-LR was not detected in the dialysate during weekly monitoring after the first detection, the authors suggested that the patients were not continuously exposed to the toxin and that the toxin detected in the serum after eight weeks may have been present in the form of bound toxin in the liver (Soares et al. 2005). Results were consistent with a mild to moderate mixed liver injury (Hilborn et al. 2013). Although the patients in the study had pre-existing diseases, the direct intravenous exposure to dialysate prepared from surface drinking water supplies put them at risk for cyanotoxin exposure and resultant adverse effects (Hilborn et al. 2013).

Cylindrospermopsin

No epidemiological studies were identified 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. However, cyanobacteria, microcystins, and other cyanotoxins were also present. As mentioned earlier, the results reported from the outbreaks should not be interpreted as cause and effect.

Human data on oral toxicity of cylindrospermopsin are limited, but results indicate that kidney and liver exhibit adverse effects due to cylindrospermopsin exposures. Information on the human health effects of cylindrospermopsin based on epidemiological studies related to drinking water are discussed in detail in the EPA's HESD for cylindrospermopsin (U.S. EPA 2015c). This information is summarized in the paragraphs that follow.

Reports of a hepatoenteritis-like outbreak (mostly in children) in Palm Island, Australia, in 1979 were attributed to consumption of drinking water with a bloom of *Cylindrospermopsis raciborskii*, a cyanobacteria that can produce cylindrospermopsin. No data are available on exposure levels or potential co-exposures to other cyanobacterial toxins and microorganisms. The majority of the cases, mostly children, required hospitalization. The clinical picture included fever, headache, vomiting, bloody diarrhea, hepatomegaly, and kidney damage with loss of water, electrolytes, and protein (Byth 1980; Griffiths and Saker 2003).

Dermal exposure to cylindrospermopsin was evaluated using skin-patch testing in humans (Pilotto et al. 2004; Stewart et al. 2006a). Exposed individuals showed mild irritation, but no statistically significant

dose-response relationship or reaction rates were found between skin reactions and increasing cell concentrations for either whole or lysed cells (Pilotto et al. 2004). No detectable skin reactions were observed in individuals exposed to lyophilized *Cylindrospermopsis raciborskii* (Stewart et al. 2006a).

5.1.1.3 Mode of Action for Noncancer Health Effects

Microcystins

Mechanistic studies have shown the importance of membrane transporters for systemic uptake and tissue distribution of microcystins by all exposure routes (Feurstein et al. 2010; Fischer et al. 2005). The importance of the membrane transporters to systemic uptake and tissue access is demonstrated by studies where there was either no liver damage or reduced damage when the hepatic organic anion transporting polypeptide (OATP) receptors were inhibited (Hermansky et al. 1990a, 1990b; Thompson and Pace 1992). OATPs are a transporter family that controls uptake of microcystins by the liver (Fischer et al. 2005).

The uptake of microcystins causes protein phosphatase inhibition and a loss of coordination between cytoskeletal protein phosphorylation by kinases and dephosphorylation by phosphatases. 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 organization of the cytoskeleton, metabolic processes, gene regulation, cell cycle control, transport and secretory processes, 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 proteins, an increase in cellular reactive oxygen species (ROS) leads to cellular apoptosis. In both in vitro and in vivo studies, cellular pro-apoptotic Bax and Bid proteins increased whereas 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 and 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. Following intraperitoneal injection of 55 μg/kg of body weight microcystin-LR, the levels of hepatic ROS increased within 0.5 hours of treatment 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.

In vitro and in vivo studies showed that cylindrospermopsin can inhibit hepatic protein synthesis (Froscio et al. 2003; Froscio et al. 2008; Terao et al. 1994), which could impact mouse urinary protein production leading to decreased urinary excretion of these proteins. 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 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) and Sukenik et al. (2006) reports, 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 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 in Humpage and Falconer (2002, 2003) are also indicative of an effect on the kidney. Numerous signs of renal damage including proteinuria, glycosuria, and hematuria were also observed in humans after a hepatoenteritis-like outbreak in Palm Island, Australia, in 1979 (Byth 1980). The outbreak was attributed to consumption of drinking water from source waters with a bloom of *Cylindrospermopsis raciborskii*. These effects have been shown to be related to impaired kidney function (Byth 1980); however, no mode of action information for kidney effects was observed in the available animal or human studies of cylindrospermopsin. Because all the studies were conducted in mice, a species that excretes low-molecular-weight proteins in urine, a study is needed of cylindrospermopsin in a laboratory species that does not excrete protein in the urine 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 2005b). The human studies are limited by lack of exposure information and the uncertainty regarding whether these studies adequately controlled for confounding factors such as hepatitis B infection. No chronic cancer bioassays for microcystins in animals are available. The EPA (U.S. EPA 2005a) 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 strong epidemiological data and a chronic bioassay of purified microcystin-LR, the data do not support classifying microcystin-LR as a carcinogen.

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 in the presence of a tumor promotor indicated preneoplastic changes consistent with its having tumorigenic

activity in mice (Falconer and Humpage 2001). Following the EPA guidelines (U.S. EPA 2005a), there is inadequate information to assess carcinogenic potential of cylindrospermopsin.

5.2 Dose-response Assessment

The RfD value for microcystins used to derive this recreational AWQC or swimming advisory is described in the EPA's HESD for microcystins (U.S. EPA 2015d). The EPA identified a 28-day study in male hybrid rats by Heinze (1999) as the critical study (described in section 5.1.1). A LOAEL of 50 µg/kg/day was identified based on increased liver weight, slight to moderate liver necrosis (necrotic severity was dose-related) with hemorrhages, and increased enzyme levels, which was used to derive an RfD of 0.05 µg/kg/day. The EPA selected the study by Heinze (1999) based on the appropriateness of the study duration, the use of multiple doses, dose-related toxicological responses, and histopathological evaluations of toxicity. After 28 days of exposure, rat organ weights (liver, kidneys, adrenals, thymus, and spleen) were measured, and hematology, serum biochemistry, and histopathology of liver and kidneys were evaluated. The critical effect in the Heinze (1999) study was supported by additional acute and subchronic data as described in the EPA's HESD for microcystins and summarized in section 5.1.1.1. The EPA's selection of uncertainty factors and derivation of the RfD are documented in its HESD for microcystins (U.S. EPA 2015d).

The RfD value for cylindrospermopsin used to derive the AWQC and swimming advisory is described in the EPA's HESD for cylindrospermopsin (U.S. EPA 2015c). The EPA identified an 11-week study in mice by Humpage and Falconer (2002, 2003) as the critical study for development of the RfD. The NOAEL was 30 µg/kg/day dose for increases in relative kidney weight seen at the LOAEL of 60 µg/kg/day. Increased relative kidney weights was the critical effect on which to base the point of departure. The EPA's selection of UFs and derivation of the RfD are documented in its HESD for cylindrospermopsin (U.S. EPA 2015c).

6.0 RECOMMENDED RECREATIONAL CRITERIA AND SWIMMING ADVISORY DERIVATION

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

6.1 Microcystins Magnitude

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

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

Where:

RfD (μg/kg/day)	=	0.05 μg/kg/day (U.S. EPA 2015d)
BW (kg)	=	31.8 kg (mean body weight of children six to 10 years; U.S. EPA 2011)
IR (L/day)	=	0.21 L/day (90th percentile daily recreational water incidental ingestion rate for children age six to 10 years; Appendix E; U.S. EPA 2018a; U.S. EPA 2011; see section 4.2.3.1)

$$\text{Microcystins recommended recreational value} = 0.05\ \mu g/kg/day \times \frac{31.8\ kg}{0.21\ L/day} = 8\ \mu g/L$$

6.2 Cylindrospermopsin Magnitude

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

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

Where:

RfD (μg/kg/day)	=	0.1 μg/kg/day (U.S. EPA 2015c)
BW (kg)	=	31.8 kg (mean body weight of children six to 10 years; U.S. EPA 2011)
IR (L/day)	=	0.21 L/day (90th percentile daily recreational water incidental ingestion rate for children age six to 10 years; Appendix E; U.S. EPA 2018a; U.S. EPA 2011; see section 4.2.3.1)

$$\text{Cylindrospermopsin recommended recreational value} = 0.1\ \mu g/kg/day \times \frac{31.8\ kg}{0.21\ L/day} = 15\ \mu g/L$$

6.3 Frequency and Duration for Recreational Criteria

The frequency and duration components of a criterion describe how often and for how long a water body's conditions can exceed the magnitude and be protective of the designated use (U.S. EPA 2005c). HABs can occur naturally, but can be an uncommon event due to a convergence of climatic and other environmental factors that result in a single short-term bloom lasting days or a couple of weeks. In some cases, multiple HABs can occur in a single year. Alternatively, longer-term HABs can occur regularly in some waters lasting for a few weeks, months, or possibly all year. HABs can occur while conditions conducive to cyanobacterial proliferation exist and limit the use of the water body for primary recreation. Water bodies where a toxic HAB has occurred in the past may experience repeat occurrences of elevated toxins when bloom-promoting conditions reoccur. In some circumstances, anthropogenic inputs are identified and controlled, and the conditions that cause the bloom can be mitigated.

The EPA recognizes that a single sample above the cyanotoxin criteria magnitude does not necessarily indicate that the designated recreational use is not attained. However, when cyanotoxin concentrations exceed the criteria magnitude either in multiple short-term blooms within a year or from a single bloom that persists for an extended period within a year, and when these patterns occur in more than one year, the designated recreational use may not be attained. The frequency and duration components discussed in this section support the identification of a trend or pattern of cyanotoxin excursions that state decision makers can use to inform the evaluation of a water body. The EPA recommends that decisions on whether the designated recreational use is attained should be flexible enough to address both types of exposure patterns when patterns reoccur in more than one year (short-term blooms that occur frequently in a recreational season, or blooms that persist for an extended period during a recreational season). States may want to evaluate the pattern of bloom occurrence and toxin concentrations within and across years to determine if there is a trend toward degradation of the water quality.

The EPA's recommended criteria duration rely on the underlying toxicity data used to derive the criteria. For both toxins, animal toxicological studies consistently demonstrate adverse health effects at various dosages and relevant timeframes. See Tables 5-1 and 5-2. For microcystins, the key study (Heinze 1999) shows adverse liver effects from repeated microcystin exposures (50 and 150 μg/kg body weight) during a study duration of 28 days. Another supporting study showed similar effects (Guzman and Solter 1999). For cylindrospermopsin, the key study (Humpage and Falconer 2002, 2003) had a duration of 11 weeks. The shorter-term studies available for cylindrospermopsin (Shaw et al., 2001; Reisner et al., 2004) were not suitable for quantification due to study limitations; however, effects observed in these studies are the same or similar to the Humpage and Falconer study (2002, 2003) and occur at similar doses. The LOAEL derived from Humpage and Falconer (2002, 2003) was determined to be protective for the adverse effects observed in the shorter duration studies. For both key studies, adverse health effects were noted at the end of the study period and it is not known if those effects occurred earlier.

The criteria are based on the same science used to develop the EPA's Drinking Water Health Advisories for microcystins and cylindrospermopsin, which are 10-day advisories (U.S. EPA 2015a, 2015b). The 10-day drinking water health advisory values represent concentrations of cyanotoxins in finished drinking water below which adverse noncarcinogenic effects are not expected to result from ingestion of drinking water over a 10-day period. Following the detection and confirmation of microcystins or cylindrospermopsin in finished drinking water above the health advisory values, the EPA recommends that drinking water utilities initiate actions to reduce exposure to consumers including determining when to notify drinking water consumers who may be more susceptible to adverse outcomes (U.S. EPA 2015c). If the advisory level continues to be exceeded after 10 days, additional public health measures can be taken, including a do-not-drink and do-not-boil water advisory. Recreational water managers

have fewer options to reduce exposure to toxins in recreational waters than do drinking water treatment operators, as recreational water does not go through a treatment process.

The EPA recommends states use 10-day assessment periods over the course of a recreation season to evaluate ambient water body condition and recreational use attainment. The 10-day period links the water body assessment to the adverse health effects from ingestion of the toxins over short-term exposures, consistent with the EPA's Drinking Water Health Advisory (described in greater detail in section 5.1). Also, Cordell (2012) discussed decade-long trends in outdoor recreation activities showing a significant proportion (43 percent) of Americans visited a beach in 2005–2009, up almost 21 percent over the previous decade. Over the same timeframe, participation in swimming in lakes and streams (42 percent of the population) increased by 14 percent (Cordell 2012). Beach visitation surveys have shown that nearly half (47 percent) of the local population are regular beach users with five or more visits in a recreation season (Caldwell et al. 2013). The recommended assessment period is reasonable considering beach visitation rates for recreators living in proximity to a beach or vacationing at a beach for a week or two with daily beach visits expected. Exposure to recreational waters containing microcystins or cylindrospermopsin at or below the recommended magnitude concentrations over the short-term 10-day duration would not be expected to result in the adverse health effects discussed in section 5.

The EPA recommends that if toxin concentrations are higher than the criterion magnitude in a sample collected during a 10-day assessment period, that period should be considered an excursion from the recreational criteria. Elevated toxin concentrations can occur over hours, days, or a couple of weeks and are counted as excursions in a recreational season. A short-term HAB that does not reoccur can result in a small number of excursions of the criteria but is not expected to result in impairment of the recreational use. Such algal blooms may result from conditions that occur naturally (e.g., as a result of unusually hot conditions), but not frequently. Following an excursion (an exceedance during the 10-day assessment period), the EPA recommends increasing the monitoring frequency to better understand the temporal and spatial nature of cyanotoxin occurrence in the affected waterbody.

In some waterbodies, longer-term HABs can persist for many weeks to months with conditions conducive to cyanobacterial proliferation. This can result in many excursions of the recommended toxin values during a recreation season. The EPA recommends that when more than three excursions (an exceedance during the 10-day assessment period) occur within a recreational season and that pattern reoccurs in more than one year, it is an indication the water quality is or is becoming degraded such that the water body no longer supports the recreational use. Recreational freshwaters at lower latitudes can have longer recreational seasons compared with those waters found at higher latitudes. For those waters in more temperate areas with a recreational season of approximately 100 days (i.e., from Memorial Day to Labor Day), three excursions could translate into a maximum of 30 percent of the recreational season not supporting the designated recreational use. Surface waters in areas with longer recreational seasons can also experience conditions that can support HAB proliferation and cyanotoxin occurrence for a longer period of the year. A maximum of three excursions across a recreational season reflects seasonal dynamics and occurrence patterns of HABs within years and the potential for adverse health effects over a short-term duration of exposure (i.e., approximately 30 days).

The EPA recognizes that multiple environmental factors can cause variability in bloom formation and toxin production, and that some years may produce HABs that occur for long periods, or HABs of shorter duration that occur repeatedly throughout a single recreational season, but such events may not occur every year. Therefore, the EPA concludes that it is appropriate to consider a pattern of multiple excursions within a recreational season as well as in multiple years (i.e., more than one year) when

determining whether the use is attained. It is important to note that the years with multiple excursions do not have to be consecutive to indicate a water quality problem. The upper-bound frequency (e.g., one year out of three years) is a risk-management decision that states need to determine when developing their water quality standards (WQS). States should include in their WQS the maximum number of years a pattern of cyanotoxin excursions can occur for the recreational use to remain supported.

The EPA does not recommend using a 10-day average concentration or a rolling average to determine an excursion, consistent with available toxicity information. States have flexibility in applying the 10-day assessment period. Some may choose to use pre-defined 10-day assessment periods for water bodies with a documented history of HAB occurrence or detection of elevated levels of cyanotoxins. Another approach is to begin the 10-day assessment period upon observation of a visible bloom. However, only considering the presence of visible blooms can miss episodes of elevated toxins (Raymond 2016). States are encouraged to consider the application of the frequency and duration components to capture elevated toxin concentrations, which may or may not coincide with the general proliferation of total cyanobacteria at high densities. More information on implementation of these values as criteria is provided in technical support materials.

6.4 Frequency and Duration for Swimming Advisory

Local and state governments can use swimming advisories to provide information to recreators on their potential exposure to cyanobacteria and their toxins. Some local and state governments currently post notifications for swimmers, in the form of advisories or warnings, when a cyanobacterial bloom is reported in recreational waters or when cyanotoxin levels exceed advisory thresholds. Table B-2 in Appendix B summarizes currently available information on state cyanotoxin-related guidelines and associated actions, including the issuance of swimming advisories.

The EPA recommends that the magnitude of the swimming advisory value not be exceeded on any single day, to provide timely information for people visiting beaches. The EPA also recommends that any exceedance of the recommended magnitude result in a swimming advisory being issued until the toxin concentration falls below the recommended magnitude. By increasing the monitoring frequency at a site where a swimming advisory is issued, water resources managers may get a clearer understanding of the temporal and spatial nature of water quality that can be useful in making decisions that protect the recreational use. Increased monitoring can also help water managers decide when to remove an advisory. The EPA has published materials for recreational water body managers that describe communicating risk to the public about cyanotoxins in recreational water bodies, monitoring, and responding to HABs (U.S. EPA 2017).

6.5 Recommended Recreational Criteria and Swimming Advisory for Microcystins and Cylindrospermopsin

The magnitude, duration, and frequency are summarized in Table 6-1.

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

Application of Recommended Values	Microcystins			Cylindrospermopsin		
	Magnitude (µg/L)	Duration	Frequency	Magnitude (µg/L)	Duration	Frequency
Recreational Water Quality Criteria	8	1 in 10-day assessment period across a recreational season	More than 3 excursions in a recreational season, not to be exceeded in more than one year[b]	15	1 in 10-day assessment period across a recreational season	More than 3 excursions in a recreational season, not to be exceeded in more than one year[b]
Swimming Advisory		One day	Not to be exceeded		One day	Not to be exceeded

[a] These recommendations can apply independently within an advisory program or in WQS. States can choose to apply either or both toxin recommendations when evaluating excursions within and across recreational seasons.

[b] An excursion is defined as a 10-day assessment period with any toxin concentration higher than the criteria magnitude. When more than three excursions occur within a recreational season and that pattern reoccurs in more than one year, it is an indication the water quality has been or is becoming degraded and is not supporting its recreational use. **As a risk-management decision, states should include in their WQS an upper-bound frequency stating the number of years that pattern can reoccur and still support its recreational use.**

The recommended magnitude represents the concentration of microcystins or cylindrospermopsin that is not expected to result in adverse human health effects from short-term recreational exposure to the toxins via incidental ingestion while swimming, based on exposure to young children. The adverse health effects include liver toxicity (for microcystins) and kidney toxicity (for cylindrospermopsin) and could result from exposures to waters containing elevated levels of these toxins.

The **water quality criteria** developed by the EPA describe the magnitude, duration, and the frequency of occurrence of pollutants. HABs may be caused or exacerbated by human activities and elevated nutrient concentrations, but cyanotoxins differ from other pollutants as they are not typically discharged into a water body. The EPA developed recommended criteria for these cyanotoxins that provide a magnitude (8 µg/L microcystins or 15 µg/L cylindrospermopsin) and duration (not to be exceeded in more than three 10-day assessment periods over the course of a recreational season). The EPA expects states to make an explicit risk management decision regarding the frequency (i.e., the number of years this pattern of exceedances can occur in the waterbody) and still support its recreational use.

As a basis for issuing a **swimming advisory**, the EPA recommends a concentration of 8 µg/L microcystins or 15 µg/L cylindrospermopsin not be exceeded on a single day. This is consistent with the goal of a swimming advisory to provide prompt information to people who wish to use the water body for recreation.

7.0 EFFECTS CHARACTERIZATION

7.1 Enhanced Susceptibility

Based on the available studies in animals, individuals with liver or kidney disease may be more susceptible to health effects than the general population as 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 observed in animal studies of cylindrospermopsin 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 (i.e., a 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 and 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.

7.2 Recreational Exposure Duration

Recreational exposure data available in the literature are expressed in two primary ways: 1) the volume of water incidentally ingested during recreation (e.g., L/hr), and 2) the duration of the recreational activity (e.g., minutes of recreation per day). A daily incidental ingestion rate distribution was developed by combining these two distributions (for more information see Appendix E). The 90th percentile of the daily incidental ingestion rate distribution for children (see section 7.3) was selected for the derivation of the criteria and swimming advisories, consistent with the 2000 Human Health Methodology.

The EPA identified the following sources of data on the duration of the recreational activity: the EPA's EFH (2011); Schets et al. (2011); and DeFlorio-Barker et al. (2017) (DFB study). See Table 7-1a and Table 7-1b for summary overviews of these studies. One major difference between the studies is in the unit of exposure, reported in minutes per day in one study and minutes per swimming event in the two other studies.

Table 7-1a. Durations of Recreational Exposures in Minutes per Day

Reference	Recreational Environment	Age Group (Years Old)	Sample Size	Mean	Units
U.S. EPA *Exposure Factors Handbook* (2011)	In Outdoor Pool or Spa	1 to 4	9	85.6	minutes per day
		5 to 11	15	164.2	
		12 to 17	5	97.0	
		18 to 64	44	117.6	
		> 64	10	78.9	

Table 7-1b. Durations of Recreational Exposures in Minutes per Swimming Event[a]

Reference	Recreational Environment	Age Group (Years Old)	Sample Size	Mean	Units
Schets et al. (2011)	Freshwater	< 15 Years	1,689	79.0	minutes per event
		16+	4,123	54.0	
	Swimming Pool	< 15	1,689	81.0	minutes per event
		16+	4,123	67.5	
DeFlorio-Barker et al. (2017)	Freshwater	< 1	171	56	minutes per event
		1 to 3	1,061	66.7	
		4 to 7	1,738[b]	88.5	
		8 to 12	2,136[c]	92.9	
		13 to 18	1,855	64	
		19 to 34	5,478	45.4	
		35+	8,058	47	
	Marine	< 1	350	60.5	
		1 to 3	2,687	79.1	
		4 to 7	4,260	107.8	
		8 to 12	5,398	121.4	
		13 to 18	4,021	102	
		19 to 34	10,786	68.2	
		35+	19,745	66.9	

[a] Additional information is needed to translate minutes per event to minutes per day.

[b] Number of children ages 4–7 reported to have contact with water: 1,562.

[c] Number of children ages 8–12 reported to have contact with water: 1,901.

The EPA considered these three studies and selected the EFH for use in deriving the criteria and swimming advisories primarily because the EFH dataset represents exposures in minutes per day. Other datasets measured the duration of recreational exposure on an event basis, which require assumptions about how many recreational events occur per day to create the relevant distribution. The EPA conducted analyses comparing these datasets, as described below to evaluate the differences in the distributions given differences in sample size, and evaluated differences given different assumptions of number of events per day.

The EFH (U.S. EPA 2011) lists time spent per 24 hours in an outdoor spa or pool for different age groups (including children five to 11 years old). The EPA acknowledges that the reported sample size

for this study is small (n = 15) for the five-to-11-year-old group. Schets et al. (2011) demonstrate that time spent in swimming pools is similar to time spent in freshwater and therefore EPA concluded that these data are representative of recreational exposure in freshwater. The EFH also presents data for minutes spent "outdoors at a pool/river/lake." The EPA did not select these data as it is uncertain if this is time spent in the water, or total time "at" the location.

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 freshwater compared to adults. Schets et al. (2011) reported similar mean duration times between swimming pools and freshwater locations for children less than 15 years old (average of 81 and 79 minutes per event, respectively; upper 95 percent CI: 200 and 270 minutes per event, respectively).

The DFB study (DeFlorio-Barker et al., 2017) compiled self-reported swimming durations from epidemiological study surveys from 12 beaches in which participants were asked to estimate, in minutes, the total time they spent in the water. Parents or guardians were responsible for answering survey questions assessing exposures such as getting water in the mouth or swallowing water, on behalf of their minor children. The study results represent 2,136 children ages eight to 12 years and 1,738 children ages four to seven recreating in freshwater. Marine recreators spent more time in the water compared with freshwater recreators. The authors suggest that behaviors may have been influenced by the warmer water at most of the marine sites (California and Gulf Coast) compared with the freshwater sites in the Great Lakes.

Although not represented in Table 7-1 a or b, the EPA's OPP uses a different approach to estimate chemical exposures for children during pool swimming, for use in its SWIMODEL (U.S. EPA 2003). This model simulates short-term exposure using a high-end estimate of exposure-time per event to represent a maximum, one-time exposure. It also simulates intermediate/long-term exposure using a shorter event duration to represent an average of maximum and minimum exposures over time. Among competitive children swimmers, the short-term exposure duration used by the SWIMODEL is one hour per day for children ages six to 10 and two hours per day for children ages 11 to 15 years based on a survey of swim coaches (U.S. EPA 2003). The competitive swimming scenario (e.g., children swimming laps) is appropriate for conducting risk assessments of exposure to swimming pool chemicals. However, it is less relevant to children's recreational activities in lakes or rivers and therefore was not used in this assessment.

7.2.1 Comparison of Duration of Exposure Distributions

Because the DFB study has a much larger sample size compared to the study results reported in EPA's EFH, the EPA conducted a statistical analysis to compare the distributions of duration of exposure. Because the DFB study age groupings and the EFH age groupings do not exactly align, the EPA compared the four-to-seven and the eight-to-12 age groups from the DFB study with the five-to-11 age group presented in the EFH. Both studies include self-reported data, which are prone to recall bias. Adult recollection of their children's time spent in the water is also uncertain. However, there is no reason to believe there would be differential recall bias between the studies.

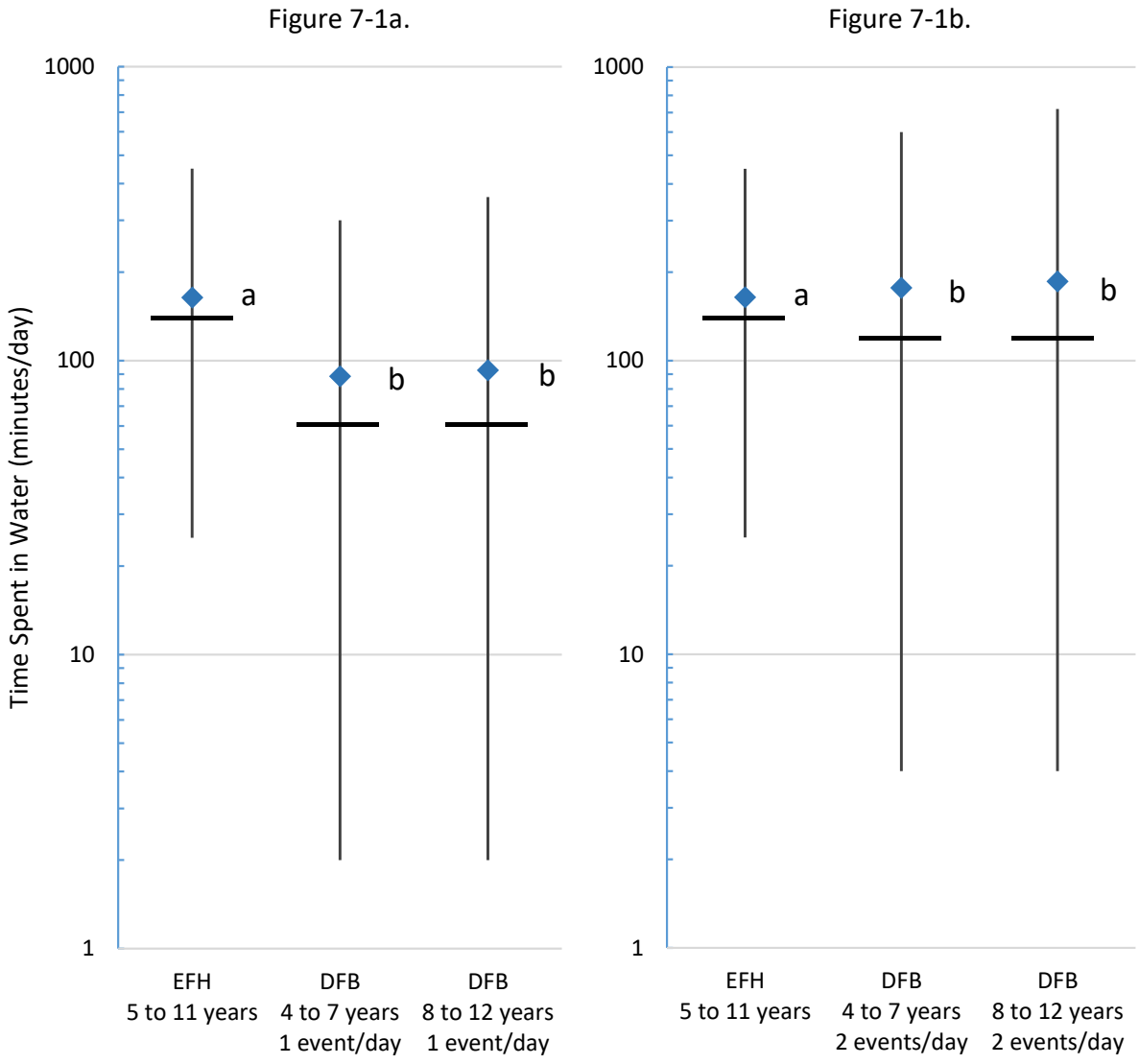
Table 7-2 shows the parameters used to create distributions for EFH and DFB studies. The EPA used assumptions of one swimming event per day and two events per day to translate the DFB duration from minutes per event to minutes per day for two different age groups. The EPA assumed the underlying distributions of exposure durations were log-normal. The observed mean and standard deviations in

Table 7-2 defined the parameters of the underlying log-normal distributions. The standard deviations take into consideration the numbers of samples, and therefore address differences in numbers of participants in the EFH and DFB studies. A large number (1 million) of samples were drawn from each log-normal distribution defined using these parameters. The distributions were truncated to reflect the observed maximum and minimum values in the EFH and DFB studies for the age groups of interest. Figures 7-1 a and b show the five resulting distributions: the EFH distribution and the DFB distributions assuming one (Figure 7-1a) and two (Figure 7-1b) events per day.

Table 7-2. Parameters Used to Fit Recreation Duration Distributions in Freshwater

Parameter Source	Age Group (sample size)	Mean (min/day)	Standard deviation	Minimum (min/day)	Maximum (min/day)
EPA 2011 EFH (minutes/day)	5 to 11 years (n = 15)	164.2	103.97	25	450
DFB 2017 (minutes/day, assuming one event/day)	4 to 7 years (n = 1,562)	88.5 (1 event)	62.8	2	300
DFB 2017 (minutes/day, assuming two events/day)		177 (2 events)	125.6	4	600
DFB 2017 minutes/day, assuming one event/day)	8 to 12 years (n = 1,901)	92.9 (1 event)	64.7	2	360
DFB 2017 minutes/day, assuming two event/day)		185.8 (2 events)	129.4	4	720

Figure 7-1 a and b. Comparison of Children's Duration of Time Spent Recreating



Comparison of children's time spent in water between EPA's 2011 *Exposure Factors Handbook* (five to 11 years old) (EFH; U.S. EPA 2011) and the DeFlorio-Barker study (DFB) (four to seven and eight to 12 years old) (DeFlorio-Barker et al. 2017) assuming one swimming event per day (Figure 7-1a) or two swimming events per day (Figure 7-1b) for the DFB data. The range of each distribution is represented by the vertical solid line, the short horizontal line indicates the median, and blue diamonds represent the mean. Letters beside the means denote significant differences of the means.

The EPA conducted two statistical tests to compare these distributions; one based on the means of the distributions and the other based on the full distributions. The full duration distribution, not the mean, in combination with the distribution of volume ingested per hour, was used to calculate the daily incidental ingestion rate. The EPA also explored how these comparisons change when one assumes that children engage in one or two swimming events per day (e.g., those who swam, took a break, and then re-entered the water at a later point in the day). The changes in the parameters are shown in Table 7-2.

For the comparison of the means, the EPA used a two-tailed t-test with unequal variances. The mean of the EFH is statistically different from both the DFB age group means (p-value < 0.001) for both one and two events per day. The means of the two DFB age groups are not statistically different from each other

(p-value = 0.08) assuming both one event and two events per day. Statistical differences between the means are denoted by letters (a and b) in Figure 7-1. Assuming two events per day for the DFB studies, the means for both DFB study age groups are significantly higher ($p < 0.001$) than the EFH mean. The larger sample size available in the DFB study results in a narrower confidence interval around the mean time spent in water, compared to the 95 percent CI for the mean used in the EFH.

For the comparison of the distributions, the EPA used the Kruskal-Wallis test. Results show that the EFH distribution is not statistically significantly different from either DFB age group distributions (p-value = 0.499, assuming one event per day; p-value = 0.498, assuming two events per day).

The EPA concluded that because the EFH and DFB distributions are not significantly different, the EFH dataset is the most appropriate for deriving criteria and swimming advisory values as it does not require additional assumptions about the number of swimming events that occur per day. The 90th percentile incidental ingestion rates are shown in Table 7-3 below for the EFH distribution and for the DFB distributions. The resulting 90th percentiles of daily incidental ingestion rate are also shown. The 90th percentile of daily ingestion rate based on the EFH distribution most closely corresponds to the 90th percentile of daily ingestion rate using the DFB dataset when two swimming events per day are assumed.

Table 7-3. Calculated Daily Incidental Ingestion Rates Based on EFH and DFB Datasets

Volume per Hour Data Source	Event Duration Data Source	Age Group (years)	Events per Day (if assumed)	90th Percentile Daily Ingestion Rate (L/day)
Recreational AWQC Appendix E full dataset (L/hr)	EPA *Exposure Factors Handbook* (2011)[a] (hr/day)	5 to 11	not needed	0.21
	DeFlorio-Barker et al. (2017) (DFB) (hr/event)	4 to 7	1	0.11
			2	0.23
		8 to 12	1	0.12
			2	0.24

[a] This distribution was used in the derivation of the criteria and recreational swimming advisories.

7.3 Evaluation of Health Protective Values for Different Lifestages

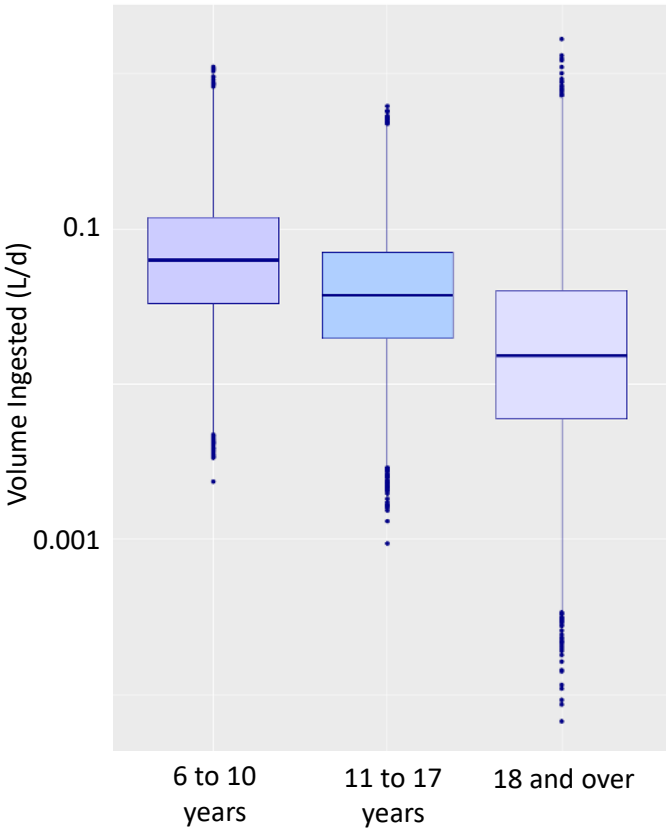
The EPA compiled and evaluated available information for various life stages before selecting children ages six to 10 years as the basis for the recreational criteria values or swimming advisory. This section discusses potential health protective values for children and adults (section 7.3.1) and focuses on exposures of younger children (less than six years) (section 7.3.2).

7.3.1 Consideration of Multiple Lifestages

The EPA used the Appendix E and the Dufour et al. (2017) dataset provided in U.S. EPA (2018a) to generate the box and whisker plots shown in Figure 7-2 for three life stages (children six to 10 years, children 11 to 17 years, and adults 18 years or older). The Appendix E Dufour data for volume ingested per swimming event was normalized to one hour. Each participant's volume ingested was adjusted to one hour based on the length of time that participant reported being in the water. The EPA converted volume of water ingested from L/event to L/hour, then used the swimming duration per day from the EPA's 2011 EFH (hours/day). The distributions were assumed to be log-normal and the plot is

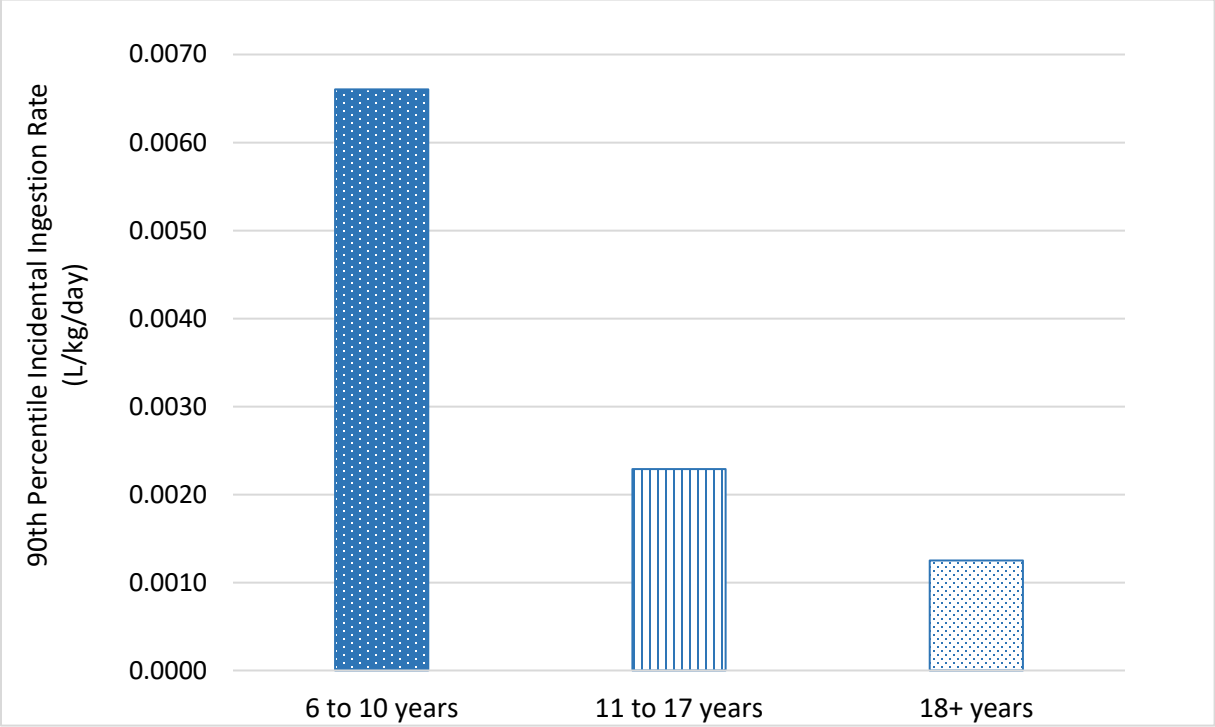
visualized in log space. The EPA used the Appendix E Dufour data on ingestion rate (shown in Figure 7-2) and the body weight estimates from the EPA's (2011) EFH (kg) to calculate the ingestion normalized by body weight (L/kg/day) shown in Figure 7-3.

Figure 7-2. Incidental Ingestion During Recreational Activity Based on Age (Appendix E)



In this box plot, the horizontal line the middle of the box is the median (Q2). The length of the box is the interquartile range (IQR) or the 25th percentile to the 50th percentile. The upper whisker vertical line extends to the greatest value less than or equal to Q3+1.5*IQR; the lower whisker extends to the smaller value less than or equal to Q1-1.5*IQR. The dots represent extreme values that are either greater than the upper whisker or lower than the lower whisker.

Figure 7-3. Comparison of Children and Adults Incidental Ingestion Rate During Recreational Activity Adjusted for Body Weight



Body weight varies by age. Table 8-1 in the EPA's EFH (U.S. EPA 2011) reported recommended statistics based on the 1999–2006 National Health and Nutrition Examination Survey. Table 7-4 shows the mean body weight for the age groups compared in this section (U.S. EPA 2011).

Table 7-4. Mean Body Weight by Age Group Based on U.S. EPA (2011)

Age Group	Body Weight (kg)
Children 6 to 10 years	31.8
Children 11 to 17 years	56.8
Adults 18 to 64	80

The EPA estimated recreational health protective values for these three different age groups for microcystins and cylindrospermopsin to demonstrate the variability due to body weight, recreational

water incidental ingestion, and exposure duration by lifestage. Inputs for these calculations are in Table 7-5.

Table 7-5. Inputs for Calculation of Protective Values for Microcystins and Cylindrospermopsin

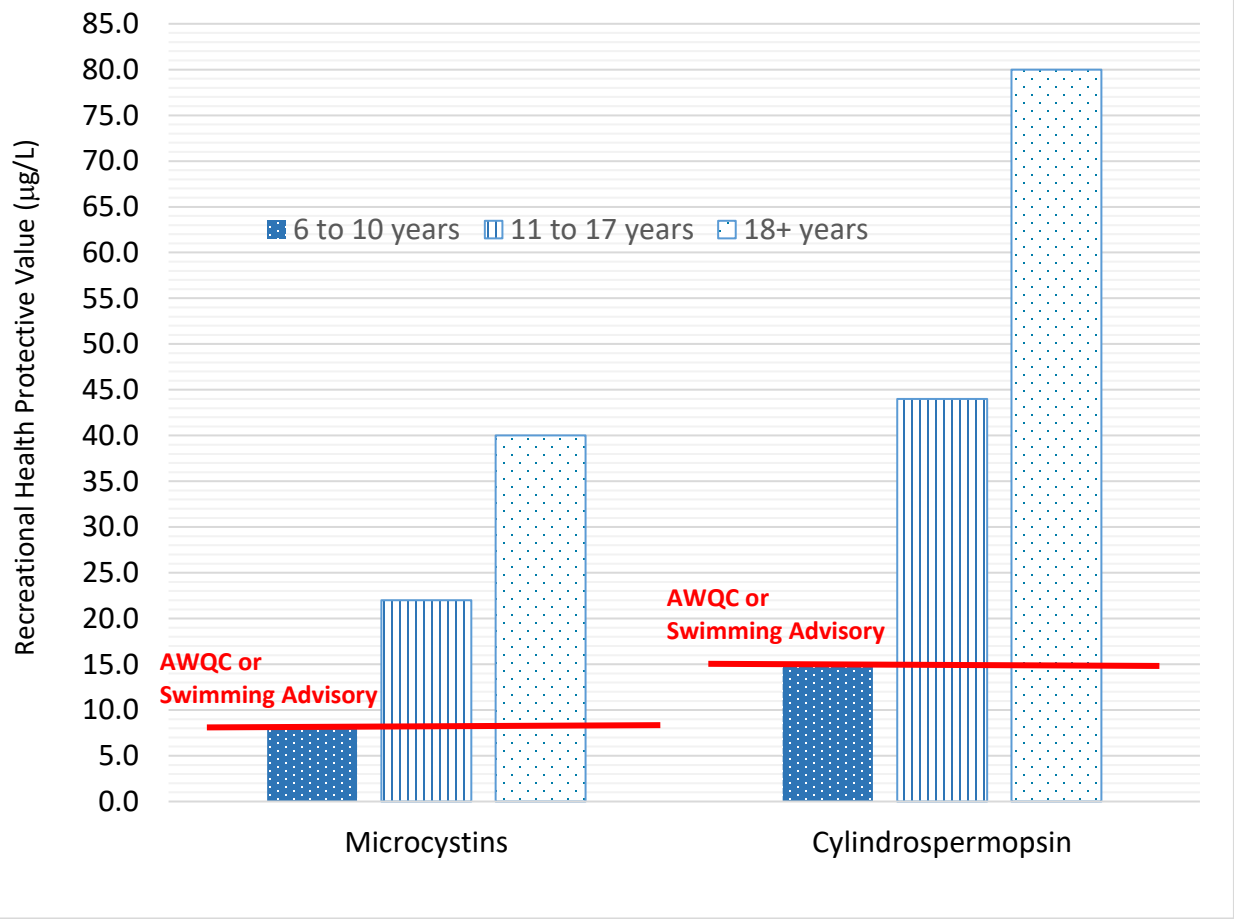
Age Group	Ingestion Rate [a] (L/day)	Body Weight [b] (kg)
Children 6 to 10 years	0.21	31.8
Children 11 to 17 years	0.13	56.8
Adults 18+ years	0.10	80.0

[a] Value is 90th percentile of the combined distribution (i.e., ingestion and duration data combined); see Appendix E.

[b] For children age 6 to 10 years, the mean body weight for the 6-to-10-year age group (31.8 kg) was used. For 11 to 17 years, the mean body weight for the 11- to 15-year-old age group (56.8 kg) was used because it was the closest age group available from the EPA's *Exposure Factors Handbook* (U.S. EPA 2011). For adults 18+ the mean body weight for the 21+ year age group (80 kg) was used (U.S. EPA 2011).

As illustrated in Figure 7-4, the AWQC and swimming advisories the EPA calculated to be protective of children ages six to 10 years are also protective of older children and adults.

Figure 7-4. Comparison of Calculated Recreational Health Protective Values for Microcystins and Cylindrospermopsin for Children, Older Children, and Adults



7.3.2 Exposure Factors for Children Younger Than Six Years Old

In the calculation of the cyanotoxin values reported in section 6, the EPA utilized exposure parameters reported in the EFH (U.S. EPA 2011) and peer-reviewed study data (study design presented in Dufour et al. 2017; data analyzed in Appendix E; U.S. EPA 2018a). The available incidental ingestion volume and exposure duration values from the Appendix E and the EPA's EFH (U.S. EPA 2011), respectively, were limited to specific age ranges. For incidental ingestion, the data reported were limited to children six years old and older because the Dufour et al. (2017) study design did not include children younger than six years. The EPA's EFH (U.S. EPA 2011) provided a mean recreational exposure duration for children ages one to four years (1.4 hour/day). This duration is shorter than the mean duration for children ages five to less than 11 years (2.7 hour/day). Values for exposure duration were not available for children younger than one year.

The EPA found one other study that characterized incidental ingestion for children. Schets et al. (2011) reported incidental ingestion volumes and durations of recreational events for children ages zero to < 15 years. However, the study did not further divide this cohort into younger children and older children. The incidental ingestion data for children < 15 years represent parental estimates of volumes of freshwater incidentally ingested by their children, which is a different methodological approach compared to the more quantitative approach used by Dufour et al. (2017). The exposure durations were also parental estimates.

The EPA calculated the 90th percentile incidental ingestion rate per day for children younger than six years old in order to compare the daily ingestion rate (L/day) between children six to 10 years and those younger than six years. The daily ingestion rate (0.21 L/day) used to derive the recreational criteria was calculated by combining the distributions for incidental ingestion and exposure duration via a probabilistic (Monte Carlo) analysis (described in section 4.2.3.1). The daily ingestion rate for children younger than six years old (0.11 L/day) was a mixed-age estimate calculated by dividing the 90th percentile for incidental ingestion for children age six to 10 years (0.077 L/hour; see Appendix E) by the mean exposure duration for children one to four years (1.4 hour/day; U.S. EPA 2011). The daily ingestion rate for children younger than six years old is lower than for children six to 10 years old. This calculation was also performed using data from Schets et al. (2011) and resulted in a daily ingestion rate of 0.1 L/day.[6] The EPA evaluated the effect of using parameter values for children younger than six years by including an age-specific body weight and the mixed-age estimate for the daily ingestion rate (L/day) parameters. Table 7-6 shows a comparison of the microcystins magnitude for the two different age groups, children ages six to 10 years and children ages one to less than six years.

The estimates for children younger than six years have large uncertainties given the lack of measured incidental ingestion data specifically for this age group. Information on exposure durations for children less than one-year-old is also lacking. Because exposure durations are greatest for five- to 11-year-olds, the EPA concluded that calculating the ingestion rate using a higher duration was protective of children younger than six years old. Research designed to fill this data gap could be helpful for characterizing the risks to children younger than six years old. Specifically, data to better characterize the volume of water ingested during recreational events would enhance EPA's confidence that the criteria values are protective of children younger than six years old.

[6] This number was calculated as follows: 0.07 L/hour (90th percentile ingestion volume for age zero to less than 15 years from Schets et al. (2011)) divided by 1.4 hr/d (mean for children one to four years from U.S. EPA 2011).

Table 7-6. Microcystins Magnitude Comparison Between Children Six to 10 and Children One to Less Than Six Years Old

Age Group	RfD (µg/kg/day)	Body Weight (kg)	Ingestion Rate (L/day)	Magnitude (µg/L)	Magnitude (µg/L) Rounded
6 to 10 years	0.05	31.8	0.21	7.57	8
1 to < 6 years	0.05	15.6 [a]	0.11	7.09	7

[a] This value is the weighted mean of the age groups one to less than two years, two to less than three years, three to less than six years (U.S. EPA 2011).

7.4 Other Recreational Exposure Pathways

The EPA selected primary contact activities and incidental ingestion of water as the primary exposure pathway for derivation of the recreational criteria and swimming advisories. Inhalation and dermal toxicity data were not available; however, there are limited available data to estimate inhalation and dermal exposure. The EPA conducted analyses to compare inhalation and dermal exposure to incidental ingestion of the cyanotoxins while recreating. Section 7.4.1 compares recreational ingestion and inhalation exposures to microcystins. Similarly, section 7.4.2 compares recreational ingestion and dermal exposure. Section 7.7 briefly discusses tribal considerations. Further research is needed to better understand the toxicity from inhalation and dermal exposure to cyanotoxins. The EPA describes the screening analyses in this section because sufficient data to quantify toxicity via these routes were not available.

7.4.1 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 molecules compared to volatile chemicals. Microcystin-associated 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).

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 where they can bind to particulate matter. Microcystins that are free or bound to particulate matter in air can be deposited into the deepest bronchiolar or alveolar cavities; air borne cyanobacterial cells from aerosolized water droplets would likely be deposited in the upper respiratory tract (Wood and Dietrich 2011).

The EPA identified field studies that measured recreators' exposure levels to aerosols containing microcystins from lakes with blooms containing microcystin-producing *Microcystis aeruginosa*. The studies found low inhalation exposures. In one study, Backer et al. (2008) used personal air samplers in a three-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. Backer et al. (2010) 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 study identified no associations between health effects and microcystin concentrations from inhalation exposure from activities that included swimming, water skiing, Jet Skiing, or boating. The authors noted that the daily mean microcystin

concentrations in personal air samples did not correlate with the concentrations of *Microcystis aeruginosa* cells, dissolved microcystins, or total microcystins in the sampled lake water.

In another study by Backer et al. (2010), the lakes had a wider range of concentrations of microcystins (< 10 to > 500 μg/L). The study authors measured microcystins exposure via personal air samplers, nasal swabs, and blood samples for individuals whose activities included swimming, boating, tubing/wakeboarding, riding watercraft, wading, and fishing at the lakes. They found low microcystin levels in personal air samplers below the limit of detection (0.1 ng/m^3) to 2.89 ng/m^3 and also in nasal swabs below the limit of detection (0.1 ng) to 5 ng. The average aerosolized microcystin concentration was approximately 0.3 ng/m^3. Based on the nasal swab data, the investigators estimated on average that the adults inhaled 0.8 ng of microcystins. Microcystin concentration in the water-soluble plasma fraction of the study subjects was also below the limit of detection (1 μg/L). The investigators cautioned that microcystin might be bound to a protein component in the blood or sequestered in liver tissue.

Wood and Dietrich (2011) studied Lake Rotorua (New Zealand) when it was experiencing a dense bloom of microcystin-producing *Microcystis*. The authors 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 at a lake with a cyanobacterial bloom. The authors measured low microcystin concentrations 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.

The EPA performed a screening analysis to characterize potential relative exposures. The EPA analyzed the relative potential dose of the cyanotoxins via inhalation exposure compared to oral ingestion to evaluate if recreational criteria values or swimming advisories based on ingestion could be protective of the other exposure routes. 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).

Using the information from Cheng et al. (2007) and inhalation exposure parameters provided in the EPA's EFH (2011), the EPA compared the estimated microcystin ingested dose to the inhaled dose. The first step in this comparative screening analysis was to calculate the incidental ingestion dose using the following equation:

$$\text{Ingestion dose (ng/day)} = \text{Ingestion rate} \times \text{Concentration}_{\text{water}}$$

Where:

Ingestion rate = 90th percentile incidental ingestion rate based on combined distributions of incidental ingestion (Appendix E) and recreational duration (U.S. EPA 2011) (L/day)

$\text{Concentration}_{\text{water}}$ = assumed concentration in water (1,000 ng/L from Cheng et al. (2007)) (ng/L)

The parameters used in the calculation of the estimated ingestion dose for each age group are presented in Table 7-7.

Table 7-7. Ingestion Parameters and Estimated Ingestion Dose for Screening-level Comparative Inhalation Exposure Analysis

Age Group	Ingestion Rate (L/day) [a]	Concentration in Water (ng/L) [b]	Ingestion Dose (ng/day)
Children	0.21	1000	210
Adults	0.10	1000	100

[a] Daily recreational incidental ingestion rate calculated in combined distribution analysis for children and adults as described in section 4.2.3.1.

[b] Cheng et al. (2007) measured 0.08 ng/m^3 in air near surface waters with a concentration of 1 µg/L microcystins. This concentration in water was assumed as part of this analysis because Cheng et al. (2007) provided aerosolized levels given a specific concentration in water.

The second step in the comparative screening analysis was to estimate the inhaled dose using the following equation:

$$\text{Inhalation dose (ng/day)} = \text{Inhalation rate} \times \text{Inhalation duration} \times \text{Concentration}_{air}$$

Where:

Inhalation rate = inhalation rate from the EPA's EFH (U.S. EPA 2011; Table 6-2) (m^3/min)

Inhalation duration = inhalation exposure duration from the EPA's EFH (U.S. EPA 2011; Table 16-20) (minutes/day)

$\text{Concentration}_{air}$ = concentration in air (0.08 ng/m^3) assumed from Cheng et al. (2007) (ng/m^3)

The inhalation exposure parameters the EPA used in this equation and the resulting estimated inhaled dose are listed in Table 7-8. The EPA selected inhalation rates for children and adults from the EPA's EFH (U.S. EPA 2011). For this conservative comparative analysis, the EPA selected the highest 95th percentile short-term, moderate intensity activity level inhalation rate—the volume of air inhaled per minute (m^3/minute)—listed for children and adults in EPA's EFH Table 6-2 "Recommended Short-

Table 7-8. Inhalation Exposure Parameters and Estimated Inhaled Dose

Age Group	Inhalation Rate (m^3/min)[a]	Duration of Inhalation Exposure per Day (minutes/day)[b]	Daily Inhalation Rate Adjusted for Duration of Exposure (m^3/day)	Concentration in Air (ng/m^3)[c]	Estimated Inhalation Dose (ng/day)
Children	0.037	560	21	0.08	1.7
Adults	0.04	511	20	0.08	1.6

[a] The EPA's *Exposure Factors Handbook* (EFH; U.S. EPA 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] Values are the longest 90th percentile duration reported for child and adult age groups in the EPA's EFH (U.S. EPA 2011) from Table 16-20 "Time Spent (minutes/day) in Selected Outdoor Locations, Doers Only, Outdoors at a Pool/River/Lake." The child and adult age groups with the longest durations spent near or in the water were children 1 to 4 years old and adults 18 to 64 years old.

[c] Cheng et al. (2007) measured 0.08 ng/m^3 in air near surface waters with a concentration of 1 mg/L microcystins.

Term Exposure Values for Inhalation (males and females combined)." The child and adult age groups with the highest of these inhalation rates were 16 to < 21 years and 51 to < 61 years, respectively.

To estimate the amount inhaled in a day, the EPA multiplied the inhalation rates for children and adults by an estimated daily inhalation exposure duration for each of these age groups. The EPA estimated daily inhalation exposure duration using a different dataset from the set it used for the incidental ingestion analysis (described in section 4.2.3.1). This was because people do not need to enter the water to be exposed via inhalation, they only need to be *near* or *at* the water. In contrast, recreators who incidentally ingest water while swimming must be *in* the water.

The EPA's EFH (U.S. EPA 2011) provides in Table 16-20 the time spent (in minutes/day) outdoors at a pool/river/lake. The EPA estimated inhalation exposure duration using the number of minutes per day spent outdoors at a pool/river/lake (U.S. EPA 2011). The EPA selected the longest 90th percentile duration values reported for child and adult age groups. The child and adult age groups with the longest times spent outdoors at a pool/river/lake were children one to four years old and adults 18 to 64 years old.

A comparison of the EPA's EFH data provided for time spent outdoors at a pool/river/lake and time spent in the water indicates that all age groups spent more time at a pool/river/lake than they spend in a pool/spa (U.S. EPA 2011). Consistent with the trend that children have longer durations of recreation in water than adults, children's time spent near recreational waters was greater than adults. The children's age group exposure patterns differed between the datasets. The data suggest younger children (one to four years) spend more time at recreational waters compared to school-aged children (five years and older), but children five to 11 years old spend more time in the water compared to other children (U.S. EPA 2011).

It is reasonable that younger children spend more time engaged in activities *at* a pool/river/lake compared to time spent recreating in recreational waters. The EPA selected this dataset to characterize inhalation exposure because younger children can spend more time playing on a beach, where they can be exposed to aerosolized cyanotoxins, than in the water where incidental ingestion can be the primary route of exposure.

The final step for this comparative screening analysis was to compare the ingestion and inhalation doses. The results are presented in Table 7-9. Using conservative assumptions for inhalation rates and inhalation exposure duration and comparing with daily incidental ingestion rates, the ingested dose is estimated to be higher than the estimated inhaled dose for children and adults. This analysis is for screening only and is highly uncertain. Further research is needed to better understand the toxicity from inhalation exposure to cyanotoxins.

Table 7-9. Results of Screening Analysis Comparing Ingestion and Inhalation Doses

Age Group	Ingestion Dose (ng/day)	Inhalation Dose (ng/day)
Children	210	1.7
Adults	100	1.6

[a] Calculations used unrounded parameters; results slightly differ with rounded values.

This analysis supports the conclusion that the inhaled dose can be much less than the incidental ingestion dose while recreating. The studies conducted by Backer et al. (2008, 2010) found low microcystin levels in aerosols above lakes with low or high microcystin concentrations and did not detect microcystin levels in the blood of study participants. In an animal study, no clinical signs or

effects on body or organ weights were observed after exposure to microcystin-LR aerosol (Benson et al. 2005). The 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 microcystins/m^3. Compared to the ingestion assumptions used for swimmers in the calculation of their recreational guideline (i.e., 50 mL/hour), CalEPA calculated that a water skier would have to inhale at least 35,000 m^3/hour 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.

Another comparison considers spray exposures from personal watercraft 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, the 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. Subjects 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 one hour, the amounts would be 1.08 mL and 11.3 mL, which are much lower than the incidental ingestion intakes per hour.

7.4.2 Dermal Absorption

The EPA did not find any peer-reviewed measured data for microcystins or cylindrospermopsin dermal absorption. The 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).

The EPA performed a comparative screening analysis to estimate the potential dermal absorbed dose of microcystins and compare it to the incidentally ingested dose. The first step in this comparative screening analysis was to calculate the incidental ingestion dose using the following equation:

$$\text{Ingestion dose} = \text{Ingestion rate} \times \text{Concentration}_{\text{water}}$$

Where:

Ingestion rate = 90th percentile incidental ingestion rate based on combined distributions of incidental ingestion (Appendix E) and recreational duration (EFH; U.S. EPA 2011) (L/day)

$\text{Concentration}_{\text{water}}$ = concentration in water assumed as the health protective value the EPA derived in this document for microcystins (mg/L)

The parameters used in the calculation of the estimated ingestion dose are presented in Table 7-10.

Table 7-10. Ingestion Parameters and Estimated Ingestion Dose for Screening-level Comparative Dermal Absorption Exposure Analysis

Ingestion Rate (L/day)[a]	Chemical Concentration in Water (mg/L)[b]	Ingestion Dose (mg/day)
0.21	0.008	0.002

[a] Daily recreational incidental ingestion rate calculated in combined distribution analysis for children and adults as described in section 4.2.3.1.

[b] Concentration in water assumed to be the health protective value for microcystins the EPA derived in this document, converted to mg/L.

To estimate the potential dermal absorbed dose, the EPA used exposure equations in its *Risk Assessment Guidance for Superfund* (U.S. EPA 2004). The first step was to use chemical-specific octanol-water partition coefficient and molecular weight values to estimate dermal permeability, a parameter needed for the equation to estimate dermally absorbed dose. Octanol-water partition coefficients 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. The EPA could not estimate cylindrospermopsin dermal absorption 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/hour)

K_{ow} = octanol-water partition coefficient from Ward and Codd (1999) (dimensionless)

MW = molecular weight (g/mole)

The chemical-specific dermal exposure parameters used to estimate skin permeability are listed in Table 7-11.

Table 7-11. Parameters Used to Estimate Skin Permeability of Microcystins

Microcystin Congener	Log K_{ow}[a]	Molecular Weight (g/mole)	Skin Permeability Coefficient (Log K_p)	Skin Permeability Coefficient (K_p) (cm/hour)
Microcystin-LR	2.16	995.17	-6.95	1.1×10^{-7}
Microcystin-LY	2.92	1002.16	-6.48	3.3×10^{-7}
Microcystin-LW	3.46	1025.2	-6.26	5.5×10^{-7}
Microcystin-LF	3.56	986.16	-5.97	1.1×10^{-6}

[a] Ward and Codd (1999)

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

$$\text{Dermal absorbed dose} = K_p \times \text{Concentration}_{water} \times t$$

Where:

Dermal absorbed dose	=	dermal absorbed dose per event (mg/cm^2-event)
K_p	=	dermal permeability coefficient of compound in water (cm/hour)
$\text{Concentration}_{water}$	=	chemical concentration in water (mg/cm^3)
t	=	event duration (hour/event)

The exposure parameters and estimated microcystins absorbed dose based on these calculations are presented in Table 7-12.

Table 7-12. Dermal Absorption Exposure Parameters and Estimated Dermal Absorbed Dose

Microcystin Congener	Chemical Conc. in Water (mg/cm^3)[a]	Event Duration[b] (hour/event) (mean for 5- to 11-year-olds)	Dermal Absorbed Dose per Event (mg/cm^2-event)	Total Body Surface Area (cm^2)[c]	Dermal Absorbed Dose per Event (mg/event)
Microcystin-LR	8×10^{-6}	2.7	2.4×10^{-12}	14,800	3.6×10^{-8}
Microcystin-LY			7.1×10^{-12}		1.0×10^{-7}
Microcystin-LW			1.2×10^{-11}		1.8×10^{-7}
Microcystin-LF			2.3×10^{-11}		3.4×10^{-7}

[a] Concentration in water assumed to be the health protective value for microcystins the EPA derived in this document, converted to mg/cm^3.

[b] Event duration is defined as time spent per day in outdoor pool or spa at home as reported in the EPA's EFH (U.S. EPA 2011).

[c] Value is 95th percentile Children 6 to 10 years from U.S. EPA (2011), converted to cm^2.

The final step for this comparative screening analysis was to compare the ingestion and dermal absorbed doses. The results are presented in Table 7-13. The estimated ingested dose is higher than the estimated dermal absorbed dose for children. This assessment is highly uncertain. Further research is needed to better understand the toxicity from dermal exposure to cyanotoxins.

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.

Table 7-13. Results of Screening Analysis Comparing Ingestion and Dermal Absorbed Doses

Microcystin Congener	Ingestion Dose (mg/day)	Dermal Absorbed Dose[a] (mg/event)
Microcystin-LR	0.002	3.6×10^{-8}
Microcystin-LY	0.002	1.0×10^{-7}
Microcystin-LW	0.002	1.8×10^{-7}
Microcystin-LF	0.002	3.4×10^{-7}

[a] Calculations used unrounded parameters; results slightly differ with rounded values.

7.5 Cyanobacterial Cells

Cyanobacteria are associated with two distinct types of stressors, as described in the conceptual model, section 4.1. The first type of stressor are the toxins (microcystins and cylindrospermopsin) produced by the cyanobacteria. Section 3 of this document discusses the nature of these stressors and section 5 discusses related health effects endpoints. These stressors are the basis of the recreational criteria and swimming advisories. The second type of stressor is cyanobacterial cells. At this time, available data are insufficient to develop quantitative recreational values for total cyanobacterial cell density related to inflammatory health endpoints. However, various state and international agencies use total cyanobacterial cell densities in decision-making to determine water quality and to post recreational warnings to the public.

Exposure to cyanobacteria cells in ambient waters is associated with numerous inflammatory health endpoints, including: rashes, respiratory and GI distress, and ear and eye irritation. These effects can be the result of direct contact with bioactive compounds in the cyanobacteria (also referred to as "endotoxins"), or by contact with cyanobacteria-associated microbial commensals via dermal, oral, or inhalation exposure routes (Eiler and Bertilsson 2004; Gademann and Portmann 2008). Section 7.5.1 and Appendix D provide more information about the health effects associated with exposure to cyanobacteria cells based on the scientific literature and related uncertainties. Section 7.5.2 presents information about the use of total cyanobacteria, or other biomass metrics, as an indicator of potential hazard associated with cells or cyanotoxins. Gene-based enumeration methods, satellite remote sensing and uncertainties related to use of cells as indicators are also described. Section 7.5.3 discusses guidelines that use total cyanobacterial cell density as an indicator for toxin presence, quantification of toxigenic cells, and an approach providing cell density estimates related to the recommended 304(a) cyanotoxin criteria.

7.5.1 Health Effects Associated with Cyanobacterial Cells and Uncertainties

Various health studies, described in more detail in Appendix D, relate recreational exposure to increasing densities of cyanobacterial cells with increased incidence of specific health endpoints that can be described as acute inflammatory or allergenic reactions. The EPA identified epidemiological studies, clinical studies, and recreational water outbreak reports in searches of the publicly available and peer-reviewed scientific literature that characterize the human health effects associated with recreating in surface waters where cyanobacteria were present (see Appendix D).

The epidemiological studies provide evidence for statistically significant associations between cyanobacterial cell densities and possible inflammatory or allergenic health endpoints:

- 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 greater than 5,000 cells/mL for more than one hour. In discussing the significance of the trend of increasing symptom occurrence and with the 5,000 cells/mL cut point, Pilotto et al. (1997) specifically suggested that the 20,000 cell/mL threshold might be too high to be adequately protective of recreators.

- Stewart et al. (2006d) found a significant increase in the inflammatory health effects associated with recreators exposed to > 100,000 total cyanobacteria/mL or a total cyanobacterial surface area > 12 mm^2/mL.

- 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 total cyanobacterial cell densities also was significant at p-value = 0.001.

- 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).

- Lévesque et al. (2016) reported a significant trend of increasing of GI illness in recreators associated with exposure to the concentration of endotoxins. The authors noted a positive correlation between endotoxin concentrations and total cyanobacterial counts. Relative risks for GI illness were higher for families that also received drinking water from the lakes studied or from wells under the influence of surface water contamination. There was no relationship between GI illness and exposure to *E. coli*. Relative risks also increased for recreators engaged in full (e.g., swimming, water skiing, diving, etc.) or limited (e.g., fishing, use of watercraft) contact recreation and adjustment for the level of exposure did not alter the health relationship.

The variability in the reported epidemiological associations in these studies in both the range of cyanobacterial cell densities reported and specific symptomologies characterized limited identification of a discrete cyanobacterial cell density value associated with a consistent level of effect. Some researchers have suggested that 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).

Scientists investigating the health effects posed by cyanobacteria have pointed out factors that contribute to the epidemiological variability observed and uncertainties in determining what level of cyanobacterial cells result in a specific level of inflammatory responses. For example:

- There are differing cyanobacterial community composition and proportions of the more allergenic, non-cyanotoxin-producing strains relative to the cyanotoxin-producing strains at each

site. Researchers have reported non-toxin-producing strains can be more allergenic compared to toxin-producing strains (Torokne et al. 2001).

- There is variability in sensitivity in the study populations.
- There are differences among the specific sites studied.
- 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 diminishes 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 complicates 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).

The number of cells in freshwater reported to be statistically-associated with a significant increase in inflammatory endpoints ranged from 5,000 to 100,000 cells per mL. The EPA concluded that, although significant associations with adverse health effects occur across a wide range of cyanobacterial cell densities, the EPA cannot derive the CWA section 304(a) criteria based on total cyanobacterial cell density at this time. There is considerable uncertainty and variability associated with the epidemiological results that did not identify consistent effects at similar cell densities and available data do not support a consistent quantitative dose-response relationship.

Additional research is needed to better describe the health effects associated with exposure to cyanobacteria with more precision using consistent health symptomologies in context with the community of cyanobacteria present (e.g., population of toxigenic versus non-toxin-producing cyanobacteria, shifts in community profile during the study, etc.) and other factors that influence the proliferation of cyanobacteria. Based on currently available science, inflammatory illnesses are significantly increased at values above 100,000 cyanobacterial cells per mL. Guideline values currently in use (see sections 2.1 and 7.5.3) that are within the 5,000 to 100,000 cell density range can find supporting scientific evidence in the peer-reviewed literature described above and in Appendix D.

7.5.2 Cyanobacteria Biomass Measurements as Indicators of Hazard

Under certain conditions, cyanobacteria possessing the toxin synthesis genes, also referred to as toxigenic cyanobacteria, begin producing cyanotoxins. Toxigenic cyanobacteria are a functional subgroup of the total cyanobacterial population that may be present in a water body and the proportion of toxigenic cells present can vary geographically and over time. Numerous biotic and abiotic factors can influence not only the dominance of cyanobacteria within the overall phytoplankton community, but also the proportion of toxigenic cyanobacteria relative to non-toxin-producing cyanobacteria (Davis et al. 2009; Hyenstrand et al. 1998; McCarthy et al. 2009; Neilan et al. 2013; Gobler et al. 2016). Multiple species of cyanobacteria are capable of producing the same toxin, such as the microcystins, which can pose a risk to human and animal health (Crawford et al. 2017). Although scientists have observed a generalized relationship between total cyanobacteria density or chlorophyll *a* and cyanotoxin concentration, these relationships are affected by the dominance of the toxin-producing cyanobacteria within the overall cyanobacterial community (Zhang et al. 2014; Loftin et al. 2016b).

Total cyanobacterial cell biomass, described by cell densities or other metrics, such as chlorophyll *a*, can function as a measure of the ecological health of a water body and as an indicator of potential public health hazards, such as inflammatory reactions from exposure to cells and adverse health effects associated with the presence of cyanotoxins. The extent, frequency, persistence, and severity of cyanobacteria proliferation can indicate the eutrophic status of a water body (Yuan and Pollard 2015). Surface water enrichment with nitrogen, notably reduced forms of nitrogen, and phosphorus have been linked to cyanobacteria becoming the dominant phytoplankton (Beaulieu et al. 2013; Glibert et al. 2016; Paerl 2008; Watson et al. 1997). Proliferating cyanobacterial biomass can result in an increased potential for toxins being produced (Pearl et al. 2001; Otten et al. 2012).

Although there can be large variation in the number of toxigenic cyanobacteria present relative to non-toxigenic cyanobacteria in any given body of water, measures of the total cyanobacterial biomass, such as cell counts, chlorophyll, or even visual assessments, can be used effectively in decision-making as early warnings of potential HAB-associated hazards (Loftin et al. 2016b). Pacheco et al. (2016) stated that these measurements can be good indicators of the potential risk of cyanotoxin exposure and useful when access to more sophisticated approaches, resources, or expertise may be limiting. Measurements of total cyanobacteria may also be particularly useful in waters with a history of HAB occurrence and the presence of elevated cyanotoxins.

7.5.2.1 Remote Sensing Techniques for Estimating Cyanotoxins

New and innovative methods, such as remote sensing techniques using satellite imagery, coupled with quantitative analysis to identify cyanobacterial blooms are of increasing interest to states. To date, these techniques cannot yet detect cyanotoxins, but they can quantify cyanobacterial densities in water bodies, an indicator of potential for cyanotoxin presence. Satellite measures of chlorophyll *a*, phycocyanin, or both are used to estimate cyanobacterial cell density based on validated algorithms that quantify relationships between these parameters and in situ measurements of cell density. For example, Stumpf (2014) and Wynne et al. (2010) readily detected by satellite areas of high *Microcystis* densities in larger freshwater bodies, such as Lake Erie.

U.S. EPA has collaborated since 2015 with the National Aeronautics and Space Administration (NASA), NOAA, and the USGS on the Cyanobacteria Assessment Network (CyAN) project. This project is developing the capability to detect and quantify total cyanobacterial blooms and related water quality of U.S. lakes and estuaries using satellite data records (U.S. EPA 2018b). This includes improving interpretation of satellite data and refining algorithms across satellite platforms. CyAN defined an approach for identifying lakes that can be spatially resolved (i.e., visually separated) with satellite imagery given differences in pixel resolutions, a method to quantify frequency of bloom occurrence in recreational freshwater sites, and a method for evaluating changes in the spatial extent of cyanobacterial blooms over time to support state-level assessments (Clark et al. 2017; Urquhart et al. 2017). CyAN has developed a mobile application that makes its processed satellite data more widely available. In 2017, the application was made available to state agencies for beta testing (U.S. EPA 2018b). A CyAN project that compares satellite-based estimations of total cyanobacterial cell density data from monitoring programs in eight states in the eastern United States found that satellite information provided robust estimates for freshwater lakes greater than 100 hectares when the cell densities less than 109,000 cells/mL and above 1 million cells/mL (Lunetta et al. 2015). The estimates were less on target for intermediate densities (i.e., between 110,000 and 1 million cells). The authors attributed this lower performance to the gap in taxonomic information needed to facilitate conversions between cell count and cell volumes (Lunetta et al. 2015).

Challenges remain for using remote sensing for cyanotoxin detection and mapping and Stumpf et al. (2016) identify these and a strategy for resolving them. The challenges they note include the lack of a steady relationship between the indicator pigments (i.e., chlorophyll *a* or phycocyanin) and cyanotoxins. These relationships may be valid for several weeks but start to vary over longer time periods due to changes in the amount of cyanotoxin produced as a function of cyanobacterial biomass. Strategic collection of pigment and toxin measurements will improve the application of remote sensing and associated models. The Ocean Land Colour Imager on the Sentinel-3 satellite, launched in 2016, will help address this need and improve data availability for most medium to large lakes around the world.

Given the inherent spatial uncertainty in the distribution of blooms and the potential issues with use of the appropriate satellite product, more attention should be given to the use of field measurements of reflectance to parameterize derivative-based pigment models (Tomlinson et al. 2016). This approach will help standardize processing of the satellite data to consistent reflectance-based products. Standardization is a factor in pigment and cyanotoxin measurement that will also require closer scrutiny. Propagation of known measurement error and uncertainty into the models will establish confidence levels for a variety of applications besides toxin maps. Improving strategies for collecting pigment measurement with toxin measurement will allow a better understanding and use of remote sensing to inform monitoring of toxins in lakes.

7.5.2.2 Molecular Methods for Estimating Cyanotoxins

Scientists have applied newer methods of quantifying microbes in environmental matrices, which increases understanding of bloom dynamics and functional subgroups of cyanobacteria, such as the toxigenic cells (Davis et al. 2009). The use of gene-based enumeration methods allows the quantification of cyanobacteria that contain specific gene sequences for toxin synthesis—without which a cell cannot produce the toxin. When toxigenic cyanobacteria are characterized with these tools, they have been shown to be better predictors of subsequent increases in toxin concentrations than with other traditional enumeration methods.

More recently, the use of gene-based quantification methods has helped to shed light on the community dynamics within a bloom, understand some of the factors that trigger toxic blooms, and provide faster and less expensive measurements of potential bloom toxicity compared to ELISA- and LC/MS/MS-based methodologies. Researchers have shown that microcystins and cylindrospermopsin are produced by non-ribosome-associated peptide synthetases (Dittmann et al. 1997; Moreira et al. 2013). The microcystin synthetase complex is encoded by 10 *mcy* genes (*mcyA* to *mcyJ*) (Neilan et al. 2013). Studies have characterized the abundance of various *mcy* genes in ambient waters (Pacheco et al. 2016; Qiu et al. 2013). The cylindrospermopsin synthetase gene cluster, *cyr*, is not as well characterized, but has been studied in multiple cylindrospermopsin-producing cyanobacteria (Neilan et al. 2013). Other researchers have used qPCR methods to characterize the relative abundance of total cyanobacteria, *Cylindrospermopsis raciborskii* and cylindrospermopsin synthase in lake water (Moreira et al. 2011). Selected examples of monitoring studies using gene-based approaches are described below.

- Davis et al. (2009) characterized toxic and nontoxic strains of *Microcystis* by quantifying the *mcyD* (toxigenic strains) and the 16S rDNA genes (all *Microcystis*) in four lakes in the northeastern United States over a two-year period. At all sites, toxigenic *Microcystis* were a better predictor of microcystin concentrations compared to total cyanobacteria, total *Microcystis*, chlorophyll *a*, or other environmental factors. Gene copies of *mcyD* were significantly correlated with microcystin concentrations in every lake studied (Davis et al. 2009).

- HABs in lakes and reservoirs are prevalent in Alberta, Canada, and are affected predominantly by elevated microcystins (Alberta Health 2014). Multiple Canadian governmental departments and public health laboratories in Alberta conducted a monitoring and advisory program for cyanobacteria at beaches. Among the findings were: microcystin-producing cyanobacteria species were dominant in most lakes with blooms peaking in late August to September, microcystin concentrations exceeding Canadian guidelines were not consistently associated with elevated total cyanobacterial cell densities in most cases, and the *mcyE* gene measured by qPCR was a good predictor for cyanobacterial blooms in some lakes (Alberta Health 2014).

- In response to the 2014 Lake Erie HAB event that contaminated the drinking water of Toledo, Ohio, the EPA revised the monitoring requirements for Ohio public water systems. Included in those requirements are testing for the *mycE* gene. If > 5 *myc*E genes/µL are detected in raw water samples, public water systems must monitor for microcystins (Ohio EPA 2017). Ohio is currently testing qPCR methods for total cyanobacteria (16s rDNA) and toxigenic cyanobacteria such as microcystin (*mcy*E gene) and saxitoxin (*sxt*A gene) producers. Ohio's HAB response strategy for recreational waters (Ohio EPA 2017) includes qPCR assessment for cyanotoxin-production genes as an option for cyanobacterial screening. If the qPCR testing indicates an abundance of toxigenic cyanobacteria, additional analysis for the toxin is recommended (Ohio EPA 2017).

- In Lake Champlain, in the northeastern United States, Fortin et al. (2015) applied qPCR-based methods and high-throughput sequencing to evaluate the effect of physico-chemical parameters and nutrients on the dynamics of cyanobacterial community. The researchers observed that total cyanobacteria were correlated with microcystin concentrations (Fortin et al. 2015). They also showed a significant correlation between the microcystin concentrations, the abundance of the *mcyD* gene, and the abundance of *Microcystis* 16S rDNA gene copies. Previous work had shown that *Microcystis* were the predominant microcystin producer present in the same water body (Ngwa et al. 2014).

- Pacheco et al. (2016) reviewed studies examining relationships between the prevalence of microcystin synthetase genes and microcystin concentration, and between chlorophyll *a* or cell density and microcystin concentration. While many studies included in the review did show a correlation for both comparisons, some did not. A lack of correlation between the synthetase genes and microcystin concentration was reported in studies that: (1) extracted the particulate-associated microcystins only; (2) included waters with very low concentrations of total microcystins (e.g., < 0.5 µg/L); or (3) in one study, monitored lakes at a single fixed point in the pelagic zone at the deepest site in each lake using depth-integrated water samples representing the entire photic zone (Beversdorf et al. 2015a; Pacheco et al. 2016). For studies not reporting a correlation between chlorophyll *a* or cell density and toxin concentration, only particulate-associated microcystin was analyzed or a very low concentration (e.g., < 0.05 µg/L) of total microcystins was observed (Pacheco et al. 2016). Zhang et al. (2014), one of the studies included in the Pacheco et al. (2016) review, characterized cylindrospermopsin- and microcystin-producing genotypes in the Macau reservoir, China, and found high cylindrospermopsin concentrations correlated to the prevalence of the *pks* gene ($r^2 = 0.95$, p-value < 0.01) and that *Cylindrospermopsis* dominated the cyanobacterial population in the reservoir studied.

- Crawford et al. (2017) applied an integrated monitoring approach including microscopic cyanobacteria identification, multiplex qPCR for toxin genes, and toxin analysis to assess

potential risks and inform bloom management decisions in a HAB event on the Murray River, Australia, in 2016. The qPCR results showed that cylindrospermopsin and saxitoxin genes were present, but were below the level of quantification. No microcystin genes were detected. The qPCR results were corroborated with the lack of detection of any cylindrospermopsin, microcystin, or saxitoxin (Crawford et al. 2017).

7.5.2.3 Uncertainties in Using Cyanobacterial Cells as Indicators

While cell density and pigment measurements can be useful for early detection of cyanobacterial proliferation and informative for bloom monitoring, these approaches may not be sufficiently accurate to predict risk from cyanotoxins (Pacheco et al. 2016). Uncertainties related to the use of total cyanobacteria in decision-making related to toxin concentrations should be considered.

1. Toxigenic cell densities can be a better indicator of the potential of a bloom to produce cyanotoxins compared to measures of total cyanobacterial biomass.

The amount of toxin produced by a toxigenic cyanobacterial cell and the relative abundance of toxigenic strains relative to non-toxigenic ones can vary considerably and be affected by environmental factors (Gobler et al. 2016). Gene-based quantification of toxigenic cyanobacteria can be beneficial for decision-making for HAB management approaches (Lee et al. 2015; Crawford et al. 2017). Davis et al. (2009) observed that quantifying toxigenic *Microcystis* was a better predictor of in situ microcystin levels than other surrogates, such as total cyanobacteria and chlorophyll *a*. The use of qPCR to characterize temporal and spatial variations in the abundance of toxigenic strains can identify the capability of a bloom to produce toxins, and hence the potential for recreator exposure to toxins, including perhaps prior to the hazardous condition occurring (Pacheco et al. 2016).

The importance of the toxigenic cyanobacterial cells has been recognized by the WHO and previously discussed in section 2.1. Based on toxigenic *Microcystis*, approximately 20 µg microcystins per L could be expected, but other species, such as *Planktothrix*, can contain higher microcystin concentrations in a cell compared to *Microcystis* (Fastner et al. 1999). Thus, the WHO commented that microcystin concentrations could be much higher (e.g., 50–100 µg/L) if species with high microcystin content dominate a bloom (WHO 2003a).

2. Total cyanobacteria can be informative as an indicator for the presence of toxins if toxigenic species are abundant or the dominant members of the cyanobacterial community.

Evidence from prior monitoring may demonstrate toxigenic strains tend to dominate blooms in a water body or that a prior bloom had increased densities of toxigenic species occurring in conjunction with elevated toxins. Studies showing good correlation between increased cell densities or other parameters linked to cell proliferation and elevated toxin concentrations can also show the bloom is dominated by toxin-producing species (Rinta-Kanto et al. 2009; Zhang et al. 2014; Pacheco et al. 2016). In one study on Lake Erie over multiple seasons, Rinta-Kanto et al. (2009) observed a positive correlation between the abundance of cyanobacterial and *Microcystis* gene copies and the number of microcystin synthetase genes. *Microcystis* were a strong contributor to the concentration of microcystins in Lake Erie and the relative abundance of *Microcystis* cells was correlated with microcystin concentrations (Rinta-Kanto et al. 2009). Lack of correlation can occur when toxigenic cell density is low or undetectable (Crawford et al. 2017) or low concentrations of toxin are recorded (Rinta-Kanto et al. 2009) and in such cases measures of total cyanobacteria are not good indicators of toxins.

3. The proliferation of toxigenic cells and the timing of the presence of elevated toxin concentrations may or may not coincide with the visible proliferation of a HAB.

Decisions to issue recreational water warnings/advisories, or initiate monitoring for cyanotoxins based on total cyanobacteria once a bloom is observed (i.e., green, discolored water, 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 or wave action, or both, or be transported downstream). A cell density of 40,000 cells/mL is lower than what might be typically associated with a visible bloom (WHO 2003a). Decision points contingent on visually confirmed blooms may miss or delay the identification of the hazardous condition associated with exposure to elevated cyanotoxins, especially in water bodies with a previous history of HAB events or toxin detections and the downstream waters potentially affected by the HAB.

Davis et al. (2009, 2010) observed bloom dominance shift between toxigenic strains and non-toxigenic strains over the course of a summer. Spatial and temporal dynamics in cyanobacterial population succession is noted in other seasonal studies (Sabart et al. 2010; Otten et al. 2012, Beversdorf et al. 2015b; Fortin et al. 2015; Chen et al. 2017). Ha et al. (2009) observed similar seasonal variations in both the gene copies of microcystin synthetase genes and for total cyanobacteria gene copies, although the cyanobacterial community was consistently dominated by microcystin-producing cells throughout the study.

7.5.3 Use of Cyanobacteria Cell Densities in Guidelines

7.5.3.1 Cyanobacteria Cell Guidelines

A number of states and international agencies include both total cyanobacteria and toxigenic cyanobacteria density guidelines to account for both inflammatory- and toxin-associated health endpoints. Cyanobacterial cell densities used by states and local health departments to provide guidance to recreators on water quality are presented elsewhere in this document (see Table 2-3 for a list of states with cyanobacterial cell density guidelines; see Appendix B for state guidelines and associated actions).

As discussed in section 2.1, the 35 states that currently have HAB-related guidelines include different approaches and guideline levels (see Table 2-3). Seven states have guideline levels that address toxin-producing cyanobacteria as a proportion of the total cyanobacterial population or include a toxin-specific cyanobacteria cell density (California, Idaho, Maryland, New York, New Hampshire, Oregon, and Virginia). The Karuk Tribe, located in California, developed cell-based values for posting cyanotoxin public health warnings for the tribe's recreational waters (Kann 2014).

As described in section 2.1 of this document, the WHO (2003a) guideline value development was informed by results from a review conducted by Chorus and Bartram (1999) and a prospective epidemiology study by Pilotto et al. (1997), which evaluated health effects after recreational exposure to cyanobacteria and reported associations between cyanobacterial cell densities and health. The WHO recommended three tiers of guideline values describing an increasing scale of potential adverse health effects and "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."

- The lowest tier of guideline values (< 20,000 cyanobacterial cells per ml; < 10 µg/L chlorophyll *a*) was mainly associated with a significant increase in irritative or allergenic effects (the inflammatory health endpoints). The WHO, using conservative assumptions, also estimated that microcystin concentrations of 2 to 4 µg/L, and possibly up to 10 µg/L, may be expected at a cell density of 20,000 cells/mL where microcystin producers dominate.

- The second tier (20,000 to 100,000 cyanobacterial cells per ml; 10 to 50 µg/L chlorophyll *a*), describing a moderate probability of adverse health effects from cyanotoxins 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. The WHO, using conservative assumptions, also estimated that 100,000 cyanobacterial cells/mL could correspond to 20 µg microcystins/L if a bloom consists of *Microcystis* and has an average microcystin content of 0.2 pg/cell.

- At the third tier (> 100,000 cells per mL; > 50 µg/L chlorophyll *a*) "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."

- Very high densities of cells occurring in scums (e.g., > 10 million cells/mL or > 5,000 µg/L chlorophyll *a*) can be associated with very high concentrations of toxin.

The Australian National Health and Medical Research Council (NHMRC) published a two-tiered guideline for managing cyanobacteria in recreational water (NHMRC 2008). Tier one includes numeric targets for microcystins based on children's recreational exposures and a toxigenic cell density for *Microcystis aeruginosa*. The NHMRC recommends a secondary guideline for the protection from health hazards associated with high densities of non-toxigenic cyanobacteria consistent with the WHO cyanobacterial cell density recommendations for the moderate probability of health effects. NHMRC used the epidemiological results published by Stewart et al. (2006b) to inform the derivation of the Australian total cyanobacteria guideline number. Stewart et al. (2006b) found a significant increase in the inflammatory health effects associated with recreators exposed to >100,000 total cyanobacteria/mL or a total cyanobacterial surface area > 12 mm^2/mL. Because different cyanobacteria species can have different sizes, the surface area estimate of biomass can take those size differences into account (e.g., 1,000 very big cells versus 1,000 very small cells). NHMRC converted the cell surface reported by Stewart et al. (2006b) to an equivalent biovolume and rounded that value to 10 mm^3/L. This biovolume guideline value applies when toxigenic cyanobacteria are absent in a bloom (NHMRC 2008)

NHMRC calculated a child-based total microcystin concentration of 9.4 µg/L, rounded to 10 µg/L (NHMRC 2008). The authors then converted the toxin concentration to an equivalent toxigenic cell density (50,000 *Microcystis aeruginosa*/mL) using the microcystin cell quota value (0.2 pg/cell). To account for the potential hazard posed by other microcystin-producing cyanobacteria, the cell density was converted into a biovolume equivalent (4 mm^3/L). Other species have different cell sizes, so the biovolume measurement allows comparisons with the other known toxin-producing cyanobacteria that may be present. The biovolume equivalent applies to the total of all cyanobacteria where a known toxin producer is dominant (NHMRC 2008).

7.5.3.2 Amount of Toxin per Cell

Toxigenic cyanobacteria produce cyanotoxins that can accumulate inside the cells or be released to the water column. The amounts of toxin produced by a toxigenic cyanobacterium is also referred to as "cell quota." There is variability in the estimate of cyanotoxin concentrations associated with cell density, in part because a bloom can contain both the toxigenic and non-toxin-producing strains of the same species and cyanobacterial community differences between locations could affect the level of cyanotoxin that is present. Thus, it is important to understand the abundance of toxigenic cyanobacteria in a water body. As discussed above, characterizing the abundance of toxin genes can be a better predictor of toxin produced than can calculations based on a toxin cell quota. The WHO's microcystin estimates at the different risk levels were based on converting the recommended total cyanobacterial cell density using a *Microcystis* cell quota value for microcystins (0.2 pg/cell) derived from a laboratory study conducted by Mole et al. (1997) reporting an average microcystin cell quota in laboratory cultures of 0.2 pg/cell (range: 0.07–0.3 pg/cell) (Fitzgerald et al. 1999), but other species and strains of microcystin producers could result in much higher water-column microcystin concentrations given the same cell density (WHO 2003a).

The EPA searched the published peer-reviewed scientific literature for information on the amount of microcystin and cylindrospermopsin produced by or contained in a cell to inform the development of toxigenic cell densities equivalent to the recommended criteria concentrations. Appendix G presents the details related to the search strategy, reference prioritization and search results. The search resulted in the collation of multiple studies reporting cell quotas for microcystin and cylindrospermopsin in multiple genera of cyanobacteria. 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 that would support conversion factors from cyanobacterial cell density (expressed in a variety of units including: cells/L, biovolume (μm^3/L), and chlorophyll *a*/L) to toxin concentrations for these species. Aggregated data are presented in Table 7-12. Table G-3 in Appendix G provides additional detail on the studies identified containing cell quota information.

To facilitate a comparison of this information with the value used by the WHO, the EPA organized the reported cell quota information by toxin and by genus (Table 7-14). Within each row, the study type, quantification method, reported means and ranges, and references to the original study are included. Not every study reported a mean, median, maximum, or minimum, so each row represents a collation of the values reported. Ranges of reported cell quotas were large. For example, for all microcystin-producing genera, reported cell quotas ranged from 0 to 4.3 pg/cell and the reported range of the means were 0.015 to 0.58 pg/cell. For *Microcystis*, the mean of the means, for seven studies published between 2008 and 2013, was 0.15 pg/cell. This value is similar to the 0.2 pg/cell value used by the WHO and provides additional evidence that this conversion factor is supported by multiple scientific studies. For the genus *Planktothrix*, the studies identified by the EPA do not suggest that this genus produces much higher amount of microcystin compared to *Microcystis*. However, the EPA's literature search focused on more recently published data and the *Planktothrix* values in the summary table come from only two recent studies that may have not characterized toxin production under optimal conditions. Based on the data presented in Table 7-14, the EPA concluded that the microcystin cell quota used by the WHO is supported. The caveat expressed by the WHO (i.e., cell quota values can be variable within and between species of microcystin-producing cyanobacteria) is also substantiated by the EPA's literature search results. The EPA included the 0.2 pg/cell value in the calculation of a toxigenic cell density for

microcystin-producing cyanobacteria equivalent to the recommended toxin magnitude (see section 7.5.3.3).

The EPA also collated similar information for cylindrospermopsin cell quotas. As with other aspects of cylindrospermopsin, less information was available, but multiple field and laboratory studies reporting the mass of toxin per cell were identified. The range of cylindrospermopsin cell quotas (0.0028–14.6 pg/cell in *Cylindrospermopsis*) was larger than for microcystins, as was the range of reported means (0.0028–0.17 pg/cell). The highest value (14.6 pg/cell) was reported from a field study (see Table G-1). The highest value reported in a laboratory study was 0.17 pg/cell. The mean value for all studies was 0.047 pg/cell (n = 10) and for field studies (n = 2) was 0.023 pg/cell. Given the few number of field studies, large uncertainties exist with how representative the mean is of the central tendency of the range. Less information was identified for *Aphanizomenon*, another well-known cylindrospermopsin producer. To have a similar confidence level in the cylindrospermopsin cell quota data compared to microcystins, additional data and an improved sense of the central tendency within the reported ranges is needed. At present, the EPA is not sufficiently confident in the cylindrospermopsin cell quota database to estimate a toxigenic cell density specific for cylindrospermopsin.

Table 7-14. Aggregated Cell Quota Summary Data for Selected Microcystin and Cylindrospermopsin-producing Genera

Toxin Genus	Quantification Method[a]; Study Type[b]	Range of Means[c]	Mean[c,d]	Median of Means[c]	Minimum; Maximum[c,e]	References
Microcystins						
All microcystin-producing genera	Mass per cell; Field and lab	0.015 pg/cell – 0.58 pg/cell	0.11 pg/cell	0.091 pg/cell	0 pg/cell – 4.3 pg/cell	Orr and Jones (1998); Jähnichen et al. (2001); Wiedner et al. (2003); Akcaalan et al. (2006); Jähnichen et al. (2007); Briand et al. (2008); Fahnenstiel et al. (2008); Vasconcelos et al. (2011); Sitoki et al. (2012); Tao et al. (2012); Wood et al. (2012); Cires et al. (2013); Sabart et al. (2013); Wang et al. (2013); Pineda-Mendoza et al. (2014); Chia et al. (2016); Wei et al. (2016)
Microcystis	Mass per cell; Field and lab	0.015 pg/cell – 0.58 pg/cell	0.11 pg/cell	0.072 pg/cell	0 pg/cell – 4.3 pg/cell	Orr and Jones (1998); Jähnichen et al. (2001); Wiedner et al. (2003); Jähnichen et al. (2007); Fahnenstiel et al. (2008); Vasconcelos et al. (2011); Sitoki et al. (2012); Tao et al. (2012); Wood et al. (2012); Cires et al. (2013); Sabart et al. (2013); Wang et al. (2013); Pineda-Mendoza et al. (2014); Chia et al. (2016); Wei et al. (2016)
	Mass per cell; Field	0.015 pg/cell – 0.58 pg/cell	0.15 pg/cell	0.075 pg/cell	0 pg/cell; 4.19 pg/cell	Fahnenstiel et al. (2008); Vasconcelos et al. (2011); Sitoki et al. (2012); Tao et al. (2012); Cires et al. (2013); Sabart et al. (2013); Wang et al. (2013)
Planktothrix	Mass per cell; Field and lab	0.076 pg/cell – 0.24 pg/cell	0.12 pg/cell	0.10 pg/cell	0.076 pg/cell; 0.24 pg/cell[e]	Akcaalan et al. (2006); Briand et al. (2008);
	Mass per cell; Field	0.091 pg/cell – 0.24 pg/cell	0.16 pg/cell	0.16 pg/cell	0.091 pg/cell; 0.24 pg/cell[e]	Akcaalan et al. (2006); Briand et al. (2008)
Fisherella	Mass per biomass; Lab	N/A	N/A	N/A	43 µg/g	Cires et al. (2014)

Toxin Genus	Quantification Method[a]; Study Type[b]	Range of Means[c]	Mean[c,d]	Median of Means[c]	Minimum; Maximum[c,e]	References
Cylindrospermopsin						
Aphanizomenon	Mass per biomass; Field and lab	N/A	N/A	N/A	7,390 µg/g; 9,330 µg/g	Yilmaz et al. (2008)
Cylindrospermopsis	Mass per cell; Field and lab	0.0028 pg/cell – 0.17 pg/cell	0.047 pg/cell	0.027 pg/cell	0.0028 pg/cell[e]; 14.6 pg/cell	Hawkins et al. (2001); Orr et al. (2010); Carneiro et al. (2013); Mohamed and Al-Shehri (2013); Davis et al. (2014); Pierangelini et al. (2015); Willis et al. (2015); Willis et al. (2016); Yang et al. (2016a)
	Mass per cell; Field	0.023 pg/cell	0.023 pg/cell	N/A	0.006 pg/cell; 14.6 pg/cell	Orr et al. (2010); Mohamed and Al-Shehri (2013)
	Mass per cell; Lab	0.0028 pg/cell – 0.17 pg/cell	0.052 pg/cell	0.031 pg/cell	0.0028 pg/cell[e]; 0.17 pg/cell[e]	Hawkins et al. (2001); Carneiro et al. (2013); Davis et al. (2014); Pierangelini et al. (2015); Willis et al. (2015); Willis et al. (2016); Yang et al. (2016a)
	Mass per biovolume; Lab	N/A	N/A	N/A	416 fg/µm^3; 447 fg/µm^3	Pierangelini et al. (2015)

fg = femtogram; pg = picogram; µg = microgram; N/A = not available.

[a] Various methods were used to quantify toxin quotas and quota values were presented in different forms, including toxin mass per cyanobacterial cell and toxin mass per cyanobacterial biomass.

[b] Studies were conducted in two different settings: the field (i.e., environmental) or a laboratory.

[c] Study authors reported data using multiple measurement units. When possible, the EPA converted data to the standard units of pg per cell. The EPA did not identify appropriate conversion factors that would allow genus-specific conversion of quotas described in mass per biomass to mass per cell.

[d] Shows single reported mean if only one study was available or average of reported means.

[e] If reported toxin quota means from one study were the lowest or highest toxin quotas reported within a genus, then these values were listed as the minimum or maximum values, respectively, to better reflect the range of toxin quota values.

[f] *Cylindrospermopsis* is now known as *Raphidiopsis.*

Challenges with collating this information include the variable conditions under which the studies characterized toxin quotas and the various ways the toxin quota data were reported. Conditions under which the toxin quotas were studied include laboratory and field conditions, different environmental and collection-based strains included in the study, and the different environmental conditions existing at the various locations where the field studies were conducted. For the latter, information on some of the external factors affecting toxin production is summarized above to help demonstrate the complex interactions that affect not just if the toxin is produced, but also how much toxin can be produced. The various ways that toxin cell quotas were reported include: toxin mass per cell, toxin mass per unit biomass, and toxin mass per unit biovolume. When possible, the EPA converted the cell quota information into pg per cell to enable a straightforward comparison to the WHO value.

7.5.3.3 Toxigenic Cyanobacteria Value Associated with Recommended Microcystins Criteria/Swimming Advisory

As discussed in section 7.5.3.2 the abundance of toxigenic cells in a water body affects the amount of cyanotoxin produced. The number of toxigenic cyanobacteria relative to the number of total cyanobacteria can vary in time and space. Quantifying the abundance of toxigenic cyanobacteria is a better predictor of potential toxin production compared to total cyanobacteria. Below, the EPA presents a similar approach to that used by the WHO to calculate a cyanobacterial cell density corresponding to recommended criteria/ swimming advisory value for microcystins. Because more data are available for microcystins compared to cylindrospermopsin, this calculation is based on microcystins only.

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

Where:

CCD	=	calculated toxigenic 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 microcystins-producing cyanobacteria (e.g., *Microcystis*):

ACC	=	8 µg/L; recommended recreational criteria value for microcystins
CTA	=	0.2 pg/cell; reported mean concentration of microcystin in a cell of microcystin-producing cyanobacteria

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

$$\text{CCD} = \frac{\text{ACC (µg/L)} \times 10^6 \text{ pg/µg}}{\text{CTA (0.2 pg/cell)}} \times \frac{\text{L}}{1000 \text{ mL}}$$

Adding in the values,

$$\text{CCD} = \frac{8\ \mu\text{g/L} \times 10^6\ \text{pg/}\mu\text{g}}{0.2\ \text{pg/cell}} \times \frac{1\ \text{L}}{1000\ \text{mL}} = 40{,}000\ \text{cells/mL}$$

Thus, a toxigenic microcystin-producing cell density of 40,000 cells/mL has the potential to result in a microcystin concentration of 8 μg/L.

7.6 Other Sources of Microcystins and Cylindrospermopsin

Although the EPA is not including other sources of cyanotoxins in this recreational exposure scenario, the Agency has included summary information on potential sources of cyanotoxins, such as drinking water, ground water, fish, shellfish, dietary supplements, air, soil, and sediments. Exposure to cyanotoxins in finished drinking water is characterized in the Drinking Water Health Advisories (U.S. EPA 2015a, 2015b). States may wish to consider these other sources of cyanotoxins in their public health approach.

7.6.1 Drinking Water

The occurrence of cyanotoxins in drinking water depends on their levels in the raw source water and the effectiveness of treatment methods for removing cyanobacteria and cyanotoxins during the production of drinking water. The EPA has provided *Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water* to assist public drinking water systems (PWSs) that choose to develop system-specific plans for evaluating their source waters for vulnerability to contamination by microcystins and cylindrospermopsin (U.S. EPA 2015e). Cyanotoxin management plan templates, water treatment optimization, and a communications tool box are also available on the EPA's Cyanotoxins in Drinking Water website (U.S. EPA 2015e).

The American Water Works Association Research Foundation (AWWARF) conducted a study on the occurrence of cyanobacterial toxins in source and treated drinking waters from 24 public water systems in the United States and Canada in 1996–1998 (AWWARF 2001). Of 677 samples tested, microcystins were found in 80 percent (539) of the waters sampled, including source and treated waters. Only two samples of finished drinking water were above 1 μg/L. A survey conducted in 2000 in Florida (Burns 2008) reported that microcystins were the most commonly found toxin in pre- and post-treated drinking water. Finished water concentrations ranged from below detection levels to 12.5 μg/L.

During the summer of 2003, a survey was conducted to test for microcystins in 33 U.S. drinking water treatment plants in the northeastern and midwestern United States (Haddix et al. 2007). Microcystins were detected at low levels ranging from undetectable (< 0.15 μg/L) to 0.36 μg/L in all 77 finished water samples.

In August 2014, the city of Toledo, Ohio, issued a do-not-drink or -boil advisory to nearly 500,000 customers in response to the presence of total microcystins in the city's finished drinking water at levels up to 2.50 μg/L. The presence of the toxins was due to a cyanobacterial bloom near Toledo's drinking water intake located on Lake Erie. The advisory was lifted two days later, after treatment adjustments led to the reduction of the cyanotoxin concentrations to concentrations below the WHO guideline value of 1 μg/L in all samples from the treatment plant and distribution system.

During the late spring and early summer of 2018, both microcystins and cylindrospermopsin were found in the finished drinking water of Salem, Oregon (Novak Consulting Group 2018). Salem's finished drinking water source is the North Santiam River, which is fed by Detroit Lake, a reservoir located southeast of the city. In late May 2018, the State of Oregon issued a recreational advisory for cyanotoxins for Detroit Lake. Less than a week later, the City of Salem issued a do not drink advisory due to the presence of levels of microcystins and cylindrospermopsin in drinking water exceeding health advisories. The drinking water advisory was lifted in the beginning of July based on many consecutive days of finished water results being below health advisory levels.

The EPA has published Drinking Water Health Advisories to address microcystins and cylindrospermopsin in drinking water (U.S. EPA 2015a, 2015b).

7.6.2 Ground Water

Only very limited data are available on microcystins in ground water and no monitoring data were identified for cylindrospermopsin. A study reported microcystins in ground water from a well located near the shore of Lake Chaohu, in China (also known as Chao Lake), which contained high microcystin concentrations (Yang et al. 2016b). Therefore, under certain conditions, ground water hydraulically connected to surface water has the potential to be contaminated by cyanotoxins.

7.6.3 Fish and Shellfish

Fish and shellfish living in waters affected by a cyanobacterial bloom may accumulate cyanotoxins in their muscle tissue and internal organs (Gibble et al. 2016; Kinnear 2010). Some authors have found that microcystins accumulate less in the edible parts of aquatic organisms, such as muscle (Deblois et al. 2011; Gutiérrez-Praena et al. 2013; Song et al. 2009; Vareli et al. 2012; Wilson et al. 2008; Xie et al. 2005; Zimba et al. 2006). Cylindrospermopsin has also been found in fish and shellfish exposed for longer periods of time to a cyanobacterial bloom (Funari and Testai 2008; Ibelings and Chorus 2007; Kinnear 2010; Saker and Eaglesham 1999). For additional information on occurrence of microcystins and cylindrospermopsin in fish and shellfish, please see the Health Advisory document published (U.S. EPA 2015a, 2015b).

7.6.4 Dietary Supplements

Extracts from *Arthrospira* (Spirulina) and *Aphanizomenon flos-aquae* have been used as dietary blue-green algae supplements (BGAS) (Funari and 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 could be contaminated with microcystins ranging from 1 μg/g up to 35 μg/g (Dietrich and Hoeger 2005). In two separate studies, Heussner et al. (2012) and Roy-Lachapelle et al. (2017) both analyzed 18 different commercially available BGAS for the presence of cyanotoxins. Heussner et al. (2012) reported that all products containing *Dolichospermum flos-aquae* (formerly *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. Roy-Lachapelle et al. (2017) reported that of the 14 products containing *Spirulina*, three contained total microcystins at levels ≤ 1 μg/g. All four products containing *Dolichospermum flos-aquae* tested positive for total microcystins ranging from 0.8 μg/g to 8.2 μg/g using the Adda oxidation method and from 0.52 μg/g to 5.8 μg/g using the sums of microcystins standards. Cylindrospermopsin was not found in any of the supplements.

7.6.5 Ambient Air

Four studies provide air concentration data for cyanotoxins indicating that recreational surface waters with toxigenic cyanobacterial blooms can result in aerosolized cyanotoxins (Backer et al. 2008, 2010; Wood and Dietrich 2011; Cheng et al. 2007). These studies are summarized in section 7.4.1.

7.6.6 Soils and Sediments

Microcystins can adsorb onto naturally suspended solids and dried crusts of cyanobacteria. Cyanotoxins can precipitate out of the water column and reside in sediments for months (Falconer 1998; Han et al. 2012; Wu et al. 2012). In sediments, cylindrospermopsin adsorbs to organic carbon, with little adsorption observed in sandy and silt sediments (Klitzke et al. 2011). The low adsorption of cylindrospermopsin in sediments/silts with low levels of organic carbon reduces the opportunity for microbial degradation.

Maghsoudi et al. (2015) tested adsorption of cyanotoxins onto three fractionated sediment particles, clay-silt (< 75 µm), fine sand (75–315 um) and coarse sand (315–2000 µm) and found that adsorption capacity of coarse sand fraction for all the tested cyanotoxins was less than four percent of the clay-silt fraction. They found that highest adsorption for cylindrospermopsin, microcystin-LW, and microcystin-LF were 73, 57, and 55 percent, respectively, and occurred within two hours. Desorption experiments demonstrated that less than nine percent of cyanotoxins desorbed from sediment within 96 hours.

Song et al. (2015) found that a statistically significant part of the variability of the microcystin concentration in the sediments could be explained by a combination of variables in the water column, such as total microcystins in the water, cyanobacterial biomass in water, pH, and temperature.

7.7 Tribal Considerations

The 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.

7.8 Livestock and Pet Concerns

The earliest observations of adverse effects of cyanobacterial exposure to animals include the rapid death of stock animals in Australia in 1878 (Francis 1878). Since then, numerous cases of mammal and bird deaths have been documented (Backer et al. 2015; Hilborn and Beasley 2015). These cases were reported throughout the 20th century on all continents except Antarctica (Stewart et al. 2008). 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).

Livestock and pets potentially can be exposed to higher concentrations of cyanotoxins, 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 also at risk, as they may lick cyanobacterial cells from their fur after swimming in a water body with an ongoing bloom (CDC 2017a). 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 microcystins/L from scum material (Chorus and 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, and in extreme cases collapse and sudden death (CDC 2017a; New York Sea Grant 2014; Trevino-Garrison et al. 2015). Although reports of livestock deaths are uncommon, 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 and Alex 1987).

The Centers for Disease Control and Prevention (CDC) provides multiple resources, such as frequently asked questions (FAQs), Veterinarian Cards, and Animal Safety Alerts, to help educate the public of the dangers associated with cyanotoxin exposure to pets (CDC 2017a, 2017b, 2017c). The CDC suggests that pet owners prevent their animals from playing in or drinking scummy water. If a dog has been swimming in scummy water, the CDC recommends rinsing them off immediately to prevent the dog from licking cyanobacteria off their fur (CDC 2017b).

The CDC recommends that pet owners contact a veterinarian if their animal shows the following symptoms of cyanotoxin poisoning: loss of appetite, loss of energy, vomiting, stumbling and falling, foaming at the mouth, diarrhea, convulsions, excessive drooling, tremors and seizures or any other unexplained sickness after being contacted with water (CDC 2017c). While there have been no HAB-associated human deaths in the United States, there have been many pet deaths (especially dogs) due to cyanotoxin exposure via swimming and ingesting contaminated waters. Overall, CDC encourages the public to follow the phrase “when in doubt, its best to keep out” (CDC 2017a).

The One Health Concept acknowledges a connection between human, animal, and environmental health, suggesting that HAB-associated animal illnesses and deaths could serve as predictors of potential HAB-associated risks in humans (CDC 2017d). Following this concept, the CDC created a voluntary reporting system called the One Health Harmful Algal Bloom System (OHHABS) (CDC 2017d). While there are other reporting systems that capture aggregate information on human illnesses or outbreaks, such as the National Outbreak Reporting System (NORS), OHHABS expands reporting to include HAB-associated environmental data, animal case data, and human case data (CDC 2017d). By collecting this information, the goal of OHHABS is to better understand HABs and HAB-associated illnesses. Members of the public can report HABs and cases of HAB-related human or animal illness by contacting local or state public health agencies (CDC 2017d).

The New York State Department of Health (NYSDOH) applied the One Health approach to implement a pilot surveillance system of HAB-related illnesses in 2015. During this pilot period, three dogs were reported to have GI symptoms after exposure to HABs in recreational water; one of these cases was also associated with a human case (Figgatt et al. 2017).

7.8.1 States and Animal HAB Guidelines

A few states have guideline levels specific to the protection of animals from cyanotoxin poisoning (Appendix H). California calculated cattle and dog action levels for the cyanotoxins microcystin and cylindrospermopsin (Butler et al. 2012). California first calculated an RfD (mg/kg body weight/day) for domestic animals for each of the cyanotoxins, based on laboratory studies. For both dogs and cattle, California estimated drinking water ingestion rates (L/kg body weight/day) based on two publications by the National Research Council, *Nutrient Requirements for Beef Cattle and Nutrient Requirements for Dogs and Cats*, and applied an UF of three to account for preferential consumption of cyanobacteria. To determine action levels (acute action level of 100 µg/L for microcystins and 200 µg/L for cylindrospermopsin), California divided the domestic animal RfD for each cyanotoxin by the final water and cyanobacterial biomass intake exposure levels calculated for cattle and dogs, and performed a unit conversion, providing a cyanotoxin concentration that would result in exposure at the RfD level or below. The state performed these calculations for an acute (lethal) and a subchronic scenario.

Oregon followed a similar approach to California's to calculate dog-specific guideline values for the cyanotoxins cylindrospermopsin, microcystin, anatoxin-a, and saxitoxin (Oregon Health Authority 2018). Oregon estimated tolerable daily intake (TDI) values for humans (µg/kg body weight/day) for each of the cyanotoxins, and applied these values to dogs (Farrer et al. 2015). Using California's dog-specific exposure estimate (L/kg body weight/day), Oregon divided the human TDI by the dog-specific ingestion rate to determine its guideline values (0.2 µg/L for microcystin and 0.4 µg/L for cylindrospermopsin).

Grayson County in Texas estimated the quantity of water that would result in a potentially lethal dose of microcystin and cylindrospermopsin for small and large dogs. Using advisory levels of 20 ppb for microcystin and cylindrospermopsin, the county calculated the volume of water that would result in a lethal or near-lethal dose of cyanotoxin by extrapolating the results of mouse studies to 10- and 80-pound dogs. This estimate 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).

At Presque Isle State Park in Pennsylvania, a HABs task force (a partnership of six agencies and organizations) monitors for microcystin and cylindrospermopsin at multiple locations on Lake Erie within the park. Some of the locations monitored include designated dog beaches. Warning signs are posted specifically for dog owners when microcystin levels are detected above 0.2 µg/L (Schnars personal communication 2017; Best personal communication 2017).

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 and Washington State Department of Health 2008; Utah Department of Environmental Quality and Department of Health 2017). Ohio includes pets in their public health advisory at threshold levels of 6 µg/L for microcystin and 5 µg/L for cylindrospermopsin; 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 (Ohio EPA 2016). Several other states including Connecticut, Idaho, Kansas, Massachusetts, Nebraska, Vermont, and Virginia provide information via pamphlets and state websites warning about harm to pets or other animals or post about harm to animals in their beach warnings and advisory signage (CDPH 2017; CDEEP 2017; 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 2018; 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): ≥ 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): ≥ 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): ≥ 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): ≥ 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): ≥ 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. • 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).

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	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.
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	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.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	(particularly where they are visibly detaching and accumulating in scum)	
	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	Second warning level: closure for public recreation.
	Cells: > 20,000 cells/ml	First 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 kindergartens, 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 kindergartens, 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.
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.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	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: ≥ 20,000 to < 100,000 cells/ml (± 20 percent)	Active daily monitoring. Counting on a weekly basis. Recreational activities are still allowed; the public is informed by posters on site.
	Microcystins: 25 µg/L (± 5 percent)	If microcystins < 25 µg/L bathing and recreational activities are restricted. If microcystins > 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: ≥ 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): ≥ 40 µg/L	Publish warnings, discourage bathing, consider temporary closure.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	Secchi Disk reading > 1 m AND microcystins: < 10 µg/L	Monitor further cyanobacterial development.
	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.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
Italy[i]	Cyanobacterial cell count for cyanotoxin-producing species other than microcystins (e.g., cylindrospermopsin, anatoxin-a) > 100,000 cells/ml (± 20 percent)	Emergency phase: weekly sampling and intensified visual inspection; quantification of all identified cyanotoxins; health surveillance; temporary bans on bathing and removal of scums from water and shoreline in addition to alert phase management measure.
	Cylindrospermopsin and anatoxin-a > 20 µg/L	Emergency phase: weekly sampling and intensified visual inspection; quantification of all identified cyanotoxins; health surveillance; temporary bans on bathing and removal of scums from water and shoreline in addition to alert phase management measures.
	Microcystin-LR: > 20 µg/L equivalents	Emergency phase: weekly sampling and intensified visual inspection; quantification of all identified cyanotoxins; health surveillance; temporary bans on bathing and removal of scums from water and shoreline in addition to alert phase management measures.
	Total cyanobacterial cell count > 20,000 cells/ml (± 20 percent) AND microcystin-LR < 20 µg/L equivalents	Alert phase: weekly sampling and visual inspection every 2 days; assessment of bloom extent and stretches of coastline affected; identify presence of cyanotoxins other than microcystins (when relevant); management measures put in place to inform citizens and prevent hazardous exposures using informative and warning panels/signs at waterfront and/or at beach access points, newsletters, brochures, publications on regional and national websites, local information systems, social network, and a Ministry toll-free number.
	Transparency ≥ 1 m AND total phosphorus < 20 µg/L	Routine phase 1: monthly sampling.
	Transparency ≥ 1 m AND total phosphorus > 20 µg/L AND total cyanobacterial cell count ≤ 2,000 cells/ml	Routine phase 2: monthly sampling and weekly visual inspection.
	Transparency ≤ 1 m AND total phosphorus > 20 µg/L AND total cyanobacterial cell count > 2,000 to < 20,000 cells/ml (± 20 percent)	Routine phase 3: fortnightly sampling and weekly visual inspection.
	Visible surface scum	Emergency phase: weekly sampling and intensified visual inspection; quantification of all identified cyanotoxins; health surveillance; temporary bans on bathing and removal of scums from water and shoreline in addition to alert phase management measures.
Netherlands[c]	Biovolume (cyanobacterial cell count): > 0 to < 2.5 mm^3/L	Surveillance level: continue fortnightly monitoring

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	Biovolume (cyanobacterial cell count): > 15 mm^3/L (if 80 percent dominance of microcystin producers and microcystins < 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. • 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.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	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. • Notify the public health unit. • Multiple sites should be inspected and sampled.
	Cyanobacteria (total): $\geq$ 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): $\geq$ 10 mm^3/L (total biovolume of all cyanobacterial material	Action (red mode) situation 2:

Jurisdiction	Recreational Water Guideline Level	Recommended Action
	where the cyanobacterial population has been tested and shown not to contain known toxins)	• 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): $\geq$ 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.
	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*: $\leq$ 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.

Jurisdiction	Recreational Water Guideline Level	Recommended Action
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.
	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. and F.N. Spon, Chapman, and 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.

[i] Funari, W; Manganelli, M; Buratti, FM; Testai, E. (2017). Cyanobacteria blooms in water: Italian guidelines to assess and manage the risk associated to bathing and recreational activities. *Science of the Total Environment, 598*, 867-880.

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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. Online searches for state guidance were conducted in 2015, 2016, and 2018. Direct personal communication of state guidelines and state public comments on the draft AWQC revealed some updated information.

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	5 states: Arizona, 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	4 states: Illinois, Iowa, Nebraska, Ohio	State guidelines address four cyanotoxins in order from most to least common: microcystins (24 states) anatoxin-a (11 states) cylindrospermopsin (9 states) saxitoxin (5 states)
Quantitative guidelines for cyanotoxins and either quantitative or qualitative guidelines for cyanobacteria	20 states: California, Colorado, Connecticut, Indiana, Kansas, Kentucky, Maryland, Massachusetts, Michigan, New Jersey, New York, North Dakota, Oklahoma, Oregon, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington	
Qualitative guidelines only	6 states: Delaware, Florida, Missouri, Montana, North Carolina, West Virginia	Examples include: presence of surface scum visible discoloration presence of potentially toxic algae presence/absence test for microcystins
Guidelines under development	4 states: Arkansas, Georgia, Minnesota, Wyoming	

Note: The EPA found that Texas and North Carolina published guidelines in the past, but the guidelines are no longer found on their websites. Missouri is in the process of developing quantitative thresholds.

Table B-2. 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: 11/27/2018.
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.healthandwelfare.idaho.gov/Portals/0/Health/EnvironmentalHealth/Idaho%20Blue-Green%20Algae%20Response%20PlanFinal.pdf. Last Accessed: 11/27/2018.
	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: 11/27/2018.
New Hampshire	Cyanobacteria: > 50 percent of total cell counts from toxigenic cyanobacteria OR the cyanobacteria cell count is greater than 70,000 cells per ml of water	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: 11/27/2018.
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: 11/27/2018. Wisconsin Department of Health Services (2016). Harmful Algal Blooms Toolkit: A Planning Guide for Public Health and Emergency Response Professionals. https://www.dhs.wisconsin.gov/publications/p0/p00853.pdf. Last Accessed: 11/27/2018.
	Visible scum layer	Post health advisory and possible beach closure.	Werner M, and 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: 11/27/2018. Wisconsin Department of Health Services (2016). Harmful Algal Blooms Toolkit: A Planning Guide for Public Health and

State	Recreational Water Guideline Level	Recommended Action	Reference
			Emergency Response Professionals. https://www.dhs.wisconsin.gov/publications/p0/p00853.pdf. Last Accessed: 11/27/2018.
States with Guidelines Based on Cyanotoxin(s) Only			
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 (2013). 2013 Statewide Harmful Algal Bloom Program. https://www2.illinois.gov/epa/topics/water-quality/monitoring/algal-bloom/Pages/2013-program.aspx. Last Accessed: 11/27/2018. Illinois Environmental Protection Agency (2018). Blue-Green Algae and Harmful Algal Blooms. https://www2.illinois.gov/epa/topics/water-quality/monitoring/algal-bloom/Pages/default.aspx. Last Accessed: 12/5/2018.
Iowa	Microcystin: ≥ 20 µg/L	Warnings are posted at state park beaches.	Iowa Environmental Council (2018). Toxic Blue-Green Algae: A Threat to Iowa Beachgoers. http://www.iaenvironment.org/our-work/clean-water-and-land-stewardship/swimming-advisories. Last Accessed: 11/27/2018.
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 (2018). Fact Sheet: Precautions and facts regarding toxic algae at Nebraska Lakes.

State	Recreational Water Guideline Level	Recommended Action	Reference
			http://deq.ne.gov/NDEQProg.nsf/OnWeb/ENV042607. Last Accessed: 5/10/2019.
Ohio	Anatoxin-a: 300 µg/L	Issue no contact advisory.	Ohio EPA (2016). State of Ohio Harmful Algal Bloom Response Strategy For Recreational Waters. http://epa.ohio.gov/portals/35/hab/HABResponseStrategy.pdf. Last Accessed: 11/27/2018.
	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.	
	Microcystins: 20 µg/L	Issue no contact advisory.	
	Microcystins: 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.	
States with Guidelines Based on Cyanobacteria and Cyanotoxin(s)			
California	Anatoxin-a: detection using an analytical method that detects <1 µg/L	Caution trigger level: increase monitoring and post caution sign warning people to stay away from scum and warning people to keep pets and livestock away from water and scum.	Butler N, Carlisle J, Kaley KB, and 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: 11/27/2018. Cyanobacteria Harmful Algal Bloom Network (2016a). Appendix to the CCHAB Preliminary Changes to the Statewide Voluntary Guidance of CyanoHABs in Recreational Waters.
	Anatoxin-a: 20 µg/L	Warning tier 1: post warning sign stating that swimming is not recommended and that pets and livestock should be kept away from the water.	
	Anatoxin-a: 90 µg/L	Danger tier 2: post sign stating that there is a present danger and that people, pets and livestock should stay out of the water and away from water spray.	
	Cylindrospermopsin: 1 µg/L	Caution trigger level: increase monitoring and post caution sign warning people to stay away from scum and warning people to keep pets and livestock away from water and scum.	

State	Recreational Water Guideline Level	Recommended Action	Reference
	Cylindrospermopsin: 4 µg/L	Warning tier 1: post warning sign stating that swimming is not recommended and that pets and livestock should be kept away from the water.	http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/appendix_a.pdf. Last Accessed: 11/27/2018. Cyanobacteria Harmful Algal Bloom Network (2016b). Table 1: CyanoHAB trigger levels for human health. http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/triggers.pdf. Last Accessed: 11/27/2018.
	Cylindrospermopsin: 17 µg/L	Danger tier 2: post sign stating that there is a present danger and that people, pets and livestock should stay out of the water and away from water spray.	
	Microcystins: 0.8 µg/L	Caution trigger level: increase monitoring and post caution sign warning people to stay away from scum and warning people to keep pets and livestock away from water and scum.	
	Microcystins: 6 µg/L	Warning tier 1: post warning sign stating that swimming is not recommended and that pets and livestock should be kept away from the water.	
	Microcystins: 20 µg/L	Danger tier 2: post sign stating that there is a present danger and that people, pets and livestock should stay out of the water and away from water spray.	
	Site-specific indicators of cyanobacteria (e.g., blooms, scums, mats)	Caution trigger level: increase monitoring and post caution sign warning people to stay away from scum and warning people to keep pets and livestock away from water and scum.	
	Toxin-producing cyanobacteria: 4,000 cells/ml	Caution trigger level: increase monitoring and post caution sign warning people to stay away from scum and warning people to keep pets and livestock away from water and scum.	
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	Colorado Department of Public Health and Environment. Algae bloom risk-management toolkit for recreational waters.

State	Recreational Water Guideline Level	Recommended Action	Reference
		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.	https://www.colorado.gov/pacific/cdphe/harmful-algae-blooms . Last Accessed: 11/27/2018
	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-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.	

State	Recreational Water Guideline Level	Recommended Action	Reference
		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 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.	

State	Recreational Water Guideline Level	Recommended Action	Reference
		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 health department that bloom has ended. dii. remove “caution” sign.	
Connecticut	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); increase regular visual surveillance until conditions change; consider cautionary postings at public access points.	Connecticut Department of Public Health and Connecticut Department of Energy and Environmental Protection (CDPH and CDEEP) (2017). Guidance to Local Health Departments for Blue-Green Algae Blooms in Recreational Freshwaters. http://www.ct.gov/deep/lib/deep/water/water_quality_management/monitoringpubs/bluegreenalgaeblooms_guidanceforlhds_2017version.pdf. Last Accessed: 11/27/2018.
	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 CTDPH and CTDEEP and expand risk communication efforts; collect samples for analysis and/or increase frequency of visual assessment; POSTED BEACH CLOSURE: if public has beach access, alert water users that a blue-green	

State	Recreational Water Guideline Level	Recommended Action	Reference
		algae bloom is present; POSTED ADVISORY: at other impacted access points.	Connecticut Department of Energy and Environmental Protection (CDEEP). (2017). Comment Letter Regarding Human Health Recreational Ambient Water Quality Criteria and/or Swimming Advisories for Microcystins and Cylindrospermopsin. March 20, 2017. Docket No. EPA-HQ-OW-2016-0715. https://www.regulations.gov/docket?D=EPA-HQ-OW-2016-0715. Last accessed: 11/27/2018.
	Anatoxin-a: 80 µg/L	Issue recreation advisory.	
Indiana	Blue-green algae: 100,000 cells/ml	Issue recreation advisory.	Indiana Department of Environmental Management (2018). Blue-Green Algae: Indiana Reservoir and Lake Update. http://www.in.gov/idem/algae/. Last Accessed: 11/27/2018.
	Cylindrospermopsin: 8 µg/L	Issue recreation advisory.	
	Microcystin-LR: 20 µg/L	Close beaches.	
	Microcystin-LR: 4 µg/L	Issue recreation advisory.	
	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	Cyanobacteria: ≥ 250,000 cells/ml	Issue public health warning.	Kansas Department of Health and Environment (2015). Guidelines for Addressing Harmful Algal Blooms in Kansas Recreational Waters. http://www.kdheks.gov/algae-illness/download/HAB_policy.pdf. Last Accessed: 11/27/2018. Kansas Department of Health and Environment (2015). Harmful Algal Blooms (HABs): KDHE Agency Response Plan. http://www.kdheks.gov/algae-illness/download/HAB_response_plan.pdf. Last Accessed: 11/27/2018.
	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.	
	Blue-green algae: > 100,000 cells/ml	Issue an HAB advisory.	

State	Recreational Water Guideline Level	Recommended Action	Reference
Kentucky	Microcystins: > 20 µg/L	Issue recreational use 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: 11/27/2018.
	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.	Commonwealth of Kentucky: Department for Environmental Protection Division of Water (2015). Harmful Algal Blooms. http://water.ky.gov/waterquality/pages/HABS.aspx. Last Accessed: 11/27/2018.
Maryland	Presence of potentially toxic algae	Issue algae bloom beach alert.	Wazniak C personal communication. (2016). Regarding Maryland Department of Natural Resources Harmful Algal Bloom (HAB) Monitoring and Management SOP. Sent via email correspondence from Catherine Wazniak, Program Manager at the MD DNR, on February 22, 2016, to John Ravenscroft, U.S. EPA. Maryland Department of Natural Resources (2014). Harmful Algal Bloom Management in the Chesapeake and Coastal Bays. http://dnr.maryland.gov/waters/bay/Documents/HAB_Management.pdf. Last Accessed: 11/27/2018.
	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.	

State	Recreational Water Guideline Level	Recommended Action	Reference
Massachusetts	Blue-green algae: > 70,000 cells/ml	Post an advisory against contact with the water.	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: 11/27/2018.
	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.	Massachusetts Department of Public Health (2008). MDPH guidelines for cyanobacteria in freshwater recreational water bodies in Massachusetts. http://www.mass.gov/eohhs/docs/dph/environmental/exposure/protocol-cyanobacteria.pdf. Last Accessed: 11/27/2018.
	Microcystin: ≥20 micrograms per liter (µg/L)	Not reported.	
Michigan	Other algal toxins are at or above appropriate guidelines that have been reviewed by MDEQ-WRD	Not reported. Post advisory.	Michigan Department of Environmental Quality (2018). Algae (Harmful Algal Blooms) website http://www.michigan.gov/deq/0,4561,7-135-3313_3681_3686_3728-383630--,00.html. Last Accessed: 11/27/2018. Kohlhepp (2015) Harmful Algal Bloom Monitoring and Assessment in Michigan Waters. Michigan Department of Environmental Quality Water Resources Division. MI/DEQ/WRD-15/013. http://www.michigan.gov/documents/deq/wrd-swas-algae-HABsummary_551207_7.pdf. Last Accessed: 03/6/2018.
	Chlorophyll *a*: >30 µg/L and visible surface accumulations/scum are present, or cells are visible throughout the water column		
	Microcystins (as total including –LR and other detectable congeners): 3 µg/L		
New Jersey	Cylindrospermopsin: 8 µg/L	Post advisory.	

State	Recreational Water Guideline Level	Recommended Action	Reference
	Anatoxin-a: 27 µg/L	Post advisory.	New Jersey Department of Environmental Protection (2017). Cyanobacterial Harmful Algal Bloom (HAB) Freshwater Recreational Response Strategy. http://www.state.nj.us/dep/wms/bfbm/NJHABResponseStrategy.pdf. Last Accessed: 11/27/2018
	Cyanobacterial cell count: ≥ 20,000 cells/ml	Post advisory.	
	Visual indication of a bloom – receipt of a bloom report or digital photograph	Suspicious Bloom: DEC HABs Program staff determine if a bloom is Suspicious and whether collection of a sample is feasible or warranted.	
New York	Blue-green chlorophyll levels: ≥ 25 µg/L; OR Microscopic confirmation that majority of sample is cyanobacteria and present in bloom-like densities; OR only in absence of the previous criteria being met: microcystin ≥ 4 µg/L but less than 20 µg/L and accompanied by ancillary evidence of the presence or recent history of a bloom	Confirmed Bloom: Signs have been developed by NY State Department of Health for use at regulated swimming beaches when Local Health Department personnel or beach operators close beaches. Online summer notification provides weekly update on the number of HABs locations in New York is included in MakingWaves, the DEC email subscription.	New York State Department of Environmental Conservation (2017). Harmful Algal Blooms (HABs) Program Guide. http://www.dec.ny.gov/docs/water_pdf/habsprogramguide.pdf. Last Accessed: 11/27/2018.
	Microcystin ≥ 20 µg/L (shoreline samples only); OR microcystin ≥ 10 µg/L (open water samples only); OR known risk of exposure to anatoxin or another cyanotoxin, based on consult between DEC HABS Program and NYSDOH staff	Confirmed with High Toxins Bloom: Signs have been developed by NY State Department of Health for use at regulated swimming beaches when Local Health Department personnel or beach operators close beaches. Online summer notification provides weekly update on the number of HABs locations in New York is included in MakingWaves, the DEC email subscription.	
	Blue-green algae bloom is present AND microcystin-LR: < 10 µg/L	Issue advisory.	
North Dakota	Blue-green algae bloom is present over a significant portion of the lake AND microcystin-LR: ≥ 10 µg/L	Issue warning.	North Dakota Department of Health: Division of Water Quality (2016). Blue-green algae advisories and warnings.
	Cyanobacteria: 100,000 cell/ml	Issue advisory.	

State	Recreational Water Guideline Level	Recommended Action	Reference
			http://www.ndhealth.gov/wq/sw/habs/defaulthabs.htm. Last Accessed: 11/27/2018.
Oklahoma	Microcystin: > 20 µg/L	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: 11/27/2018.
	Anatoxin-a: ≥ 20 µg/L	Issue public health advisory.	
Oregon	Cylindrospermopsin: ≥ 20 µg/L	Issue public health advisory.	Oregon Health Authority (2018). Oregon Harmful Algae Bloom Surveillance (HABS) Program Public Health Advisory Guidelines: Harmful Algae Blooms in Freshwater Bodies. https://www.oregon.gov/oha/ph/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Documents/HABPublicHealthAdvisoryGuidelines.pdf. Last Accessed: 11/27/2018.
	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.	
	Saxitoxin: ≥ 10 µg/L	Issue public health advisory.	
	Toxigenic species: > 100,000 cells/ml	Issue public health advisory.	
	Visible scum with documentation and testing	Issue public health advisory.	
	Microcystin: > 6 µg/L	Recreational Public Health Advisory.	
Pennsylvania	Microcystin: > 20 µg/L	Recreational No Contact Advisory.	Pennsylvania Department of Environmental Protection (2014). Lake Erie Harmful Algal Bloom Monitoring and Response Strategy for Recreational Waters. https://seagrant.psu.edu/sites/default/files/PA%20Lake%20Erie%20Harmful%20Algal%20Bloom%20Response%20Strategy%20For%20Recreational%20Waters%20-%202nd%20Draft.pdf. Last Accessed: 11/27/2018.
	Cylindrospermopsin: > 5 µg/L	Recreational Public Health Advisory.	
	Cylindrospermopsin: > 20 µg/L	Recreational No Contact Advisory.	
	Anatoxin-a: > 80 µg/L	Recreational Public Health Advisory.	
	Anatoxin-a: > 300 µg/L	Recreational No Contact Advisory.	
	Saxitoxin: > 0.8 µg/L	Recreational Public Health Advisory.	
	Saxitoxin: > 3 µg/L	Recreational No Contact Advisory.	

State	Recreational Water Guideline Level	Recommended Action	Reference
	HAB verified by visual observation	Recreational no contact advisory.	
	Cyanobacteria: > 70,000 cells/ml	Issue health advisory.	
Rhode Island	Microcystin-LR: ≥ 14 µg/L	Issue health advisory.	Rhode Island Department of Environmental Management, and 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: 11/27/2018.
	Visible cyanobacteria scum or mat	Issue health advisory.	
	Anatoxin-a: detection 90 µg/L	Tier 2: Warning: Issue WARNING advisory, Post WARNING signs, sampling recommended weekly.	
Utah	Anatoxin-a: > 90 µg/L	Tier 3: Danger: Issue DANGER advisory, Post DANGER signs, consider CLOSURE, sampling recommended at least weekly.	Utah Department of Environmental Quality and Department of Health (2017). Utah HAB Guidance Summary. http://health.utah.gov/enviroepi/appletree/HAB/HAB_Guidance_Summary_2017.pdf . Last Accessed: 11/27/2018.
	Cyanobacteria: 20,000 – 10,000,000 cells/ml	Tier 2: Warning: Issue WARNING advisory, Post WARNING signs, sampling recommended weekly.	
	Cyanobacteria: >10,000,000 cells/ml	Tier 3: Danger: Issue DANGER advisory, Post DANGER signs, consider CLOSURE, sampling recommended at least weekly.	
	Microcystin: 4 – 2,000 µg/L	Tier 2: Warning: Issue WARNING advisory, Post WARNING signs, sampling recommended weekly.	
	Microcystin: > 2,000 µg/L	Tier 3: Danger: Issue DANGER advisory, Post DANGER signs, consider CLOSURE, sampling recommended at least weekly.	
	Cylindrospermopsin: > 8 µg/L	Tier 2 or 3: Consult with Utah Department of Environmental Quality and Utah Department of Health as needed on this issue.	
	Reports of animal illnesses or death	Tier 2: Warning: Issue WARNING advisory, Post WARNING signs, sampling recommended weekly.	

State	Recreational Water Guideline Level	Recommended Action	Reference
	Reports of human illness	Tier 3: Danger: Issue DANGER advisory, Post DANGER signs, consider CLOSURE, sampling recommended at least weekly.	
	Anatoxin-a: ≥ 10 µg/L	Close recreational beaches.	
Vermont	Cylindrospermopsin: ≥ 10 µg/L	Close recreational beaches.	Vermont Department of Health (2015). Cyanobacteria (Blue-green Algae) Guidance for Vermont Communities. http://www.healthvermont.gov/sites/default/files/documents/2016/12/ENV_RW_CyanobacteriaGuidance.pdf. Last Accessed: 11/27/2018.
	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.	
	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	Microcystin: > 6 µg/L	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. http://www.vdh.virginia.gov/content/uploads/sites/12/2016/02/VDHMicrocystisGuidance.pdf. Last Accessed: 11/27/2018.
	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.	
	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.	

State	Recreational Water Guideline Level	Recommended Action	Reference
Washington	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, and 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: 11/27/2018.
	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.	Hardy J, and 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: 11/27/2018.
	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.	
	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, and 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: 11/27/2018.
	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	Hardy J, and Washington State Department of Health (2011). Washington State Provisional Recreational Guidance for Cylindrospermopsin and Saxitoxin.

State	Recreational Water Guideline Level	Recommended Action	Reference
		toxicity, or reports of illness, pet death than tier 3: local health posts DANGER sign; lake closed.	http://www.doh.wa.gov/portals/1/documents/4400/332-118-cylindrosax%20report.pdf. Last Accessed: 11/27/2018.
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: Division of Water. Blue-Green Algae in Delaware. (2016). http://www.dnrec.delaware.gov/wr/INFORMATION/OTHERINFO/Pages/Blue-GreenAlgae.aspx. Last Accessed: 11/27/2018.
Florida	Cyanobacteria bloom	Issue health advisory; post warning signs.	Florida Department of Environmental Protection (2019). Freshwater Algal Blooms: Frequently Asked Questions. https://floridadep.gov/sites/default/files/freshwater-algal-bloom-faqs_2019.pdf Last Accessed: 5/10/2019.
Missouri	Microcystins: presence (test strip range 0 to 10 ng/ml) Cylindrospermopsin: presence (test strip range 0 to 10 ng/ml) Anatoxin-a: presence (test strip range 0 to 2.5 ng/ml)	Missouri has a multi-agency proactive approach to address events which can result in the decision to temporary close swim beaches and post notices regarding the bloom around the lake to protect the citizens of Missouri from the health risk posed by exposure to a HAB. Information is also released to through the news media and social media to quickly share the possible health risk with the largest audience possible.	Missouri Department of Natural Resources (2017) Qualitative screening of algal toxins in drinking water and recreational waters using strip test by Abraxas, Inc. https://dnr.mo.gov/env/docs/mdnresp360.pdf. Last Accessed: 11/27/2018. Missouri Department of Natural Resources (2018) Harmful Algal Blooms and Blue-Green Algae. Website https://dnr.mo.gov/env/cyanobacteria.htm. and http://ephtn.dhss.mo.gov/EPHTN_Data_Portal/pdf/success-stories/MO-Blue-Green-

State	Recreational Water Guideline Level	Recommended Action	Reference
			Algae-Task-Force-Establishment.pdf Last Accessed: 11/27/2018.
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: 11/27/2018.
North Carolina	Visible discoloration or surface scum	Microcystin testing.	North Carolina Health and Human Services: Division of Public Health (2014). Occupational and Environmental Epidemiology: Cyanobacteria (Blue-green Algae). http://epi.publichealth.nc.gov/oee/a_z/algae.html. Last Accessed: 11/27/2018.
West Virginia	Blue-green algal blooms observed and monitored	Issue public health advisory.	West Virginia Department of Health and 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: 11/27/2018.
States with Guidelines Under Development			
Arkansas	TBD	TBD	Arkansas Beautiful Buffalo River Action Committee (2018). https://bbrac.arkansas.gov/pdfs/201701205

State	Recreational Water Guideline Level	Recommended Action	Reference
			-arkansas-harmful-algal-bloom-(habs)-workgroup.pdf. Last Accessed: 11/27/2018.
Georgia	TBD	TBD	Georgia Department of Public Health (2018). https://www.gachd.org/programs-services/environmental-health-2/beach_water_testing/. Last Accessed: 03/6/2018.
Minnesota	TBD	TBD	Minnesota Department of Health (2015). Toxicological Summary for: Microcystin-LR. http://www.health.state.mn.us/divs/eh/risk/guidance/gw/microcystin.pdf. Last Accessed: 11/27/2018.
Wyoming	TBD	TBD	Wyoming Department of Environmental Quality (2018). Harmful Algal Bloom Website. http://deq.wyoming.gov/wqd/nutrient-pollution/resources/harmful-algal-blooms/. Last Accessed: 11/27/2018.

Note: Alabama, Alaska, Hawaii, Louisiana, Mississippi, Nevada, New Mexico, South Carolina, South Dakota, Tennessee, and Texas did not have guidelines available online. Missouri is in the process of developing quantitative thresholds.

APPENDIX C. LITERATURE SEARCH DOCUMENTATION

The recreational ambient water quality criteria (AWQC) or swimming advisories document for microcystins and cylindrospermopsin relied significantly on information identified, reviewed, and synthesized in the 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, 2015d, 2015a, 2015b). The 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 (HESDs), the EPA conducted a comprehensive literature search from January 2013 to May 2014 using Toxicology Literature Online (TOXLINE), PubMed, and Google Scholar. The 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 HESDs for microcystins and cylindrospermopsin (U.S. EPA 2015c, 2015d).

The EPA conducted supplemental literature searches in September 2015 to capture references published since the completion of the HESDs' 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 the EPA's 2015 HESDs 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 zero 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)?

The 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?

The 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, the EPA retrieved nine 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

C.1 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 the EPA's 2015 HESDs for Cylindrospermopsin and Microcystins?

The 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, the 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.

C.2 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, the 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, the 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, the 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, the 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.

C.3 Research Question 4: What new information, if any, is available regarding how aquatic recreational exposure ingestion rates in children differ among age groups between zero and 18 years?

Search of Bibliographic Databases

The 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 the EPA's (2011) *Exposure Factors Handbook* (EFH) (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, the EPA retrieved five articles, four of which were published between 2013 and 2015 and appeared to measure or estimate incidental water ingestion. The 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, the 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
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, the 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

C.4 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)?

The 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. The EPA first searched PubMed and WoS with a focus on dogs. The 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, the EPA retrieved five of the 35 journal articles retrieved during the search focused on dogs. These five 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 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.4.1 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.4.2 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.4.3 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

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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. The 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.

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 that did 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 seven 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 non-toxin-producing strain) sensitized 91 percent 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 intraperitoneal with either sonicated or live cells from a *Microcystis* water bloom, developed delayed-type hypersensitivity when challenged two 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 *Cylindrospermopsis raciborskii* solutions generated irritation of the abdominal skin exposed during induction (two percent w/v lysed cell solution containing 73 µg/mL cylindrospermopsin). Subsequent dermal exposures to the *Cylindrospermopsis 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 non-toxin 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 *Cylindrospermopsis 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 odds ratio (OR) of 2.13 and a 95 percent confidence interval (CI) of 1.79–4.21 ($p < 0.001$). Lysed cells patch analysis showed an OR of 3.41 and a 95 percent 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 percent (95 percent CI: 15–31 percent). When subjects reacting to negative controls (39) were excluded, the mean percentage was 11 percent (95 percent CI: 6–18 percent). 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 six 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: *Cylindrospermopsis raciborskii* and mixed *Microcystis aeruginosa* and *Cylindrospermopsis 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 nontoxic extracts of *Microcystis aeruginosa* in 259 patients with chronic rhinitis over two 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 nontoxic portion of the cyanobacteria.

Geh et al. (2015) studied the immunogenicity of extracts of toxic and nontoxic strains of *Microcystis aeruginosa* in patient sera (18 patients with chronic rhinitis and three 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 nontoxic *Microcystis aeruginosa* extract than the extract from the toxic strain. After pre-incubation of the nontoxic 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.

Facciponte et al. (2018) used polymerase chain reaction (PCR) to detect aerosolized cyanobacteria inhaled into the human respiratory tract. They found cyanobacteria at high frequencies in the upper respiratory tract (92.2 percent) and central airway (79.3 percent) of the study subjects (n = 77). The findings suggests that humans inhale aerosolized cyanobacteria, which can remain in the nostrils and the lungs.

D.1.3 Epidemiological Studies, Case Reports, and Outbreaks

Among the epidemiological studies discussed here, some identified significant associations between cyanobacteria exposure and a range of health outcomes including dermal, eye/ear, gastrointestinal (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. 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. Overall, these studies provide evidence of statistically 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.

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 and 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 and Bates 1992; Philipp et al. 1992), and three were conducted in subtropical 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 United Kingdom (Hampshire)	*Microcystis* sp., *Gleotrichia* sp.	–	No statistically significant health associations	No quantitative cyanobacterial cell densities provided
Philipp and Bates (1992)	Cross-sectional n = 382 United Kingdom (Somerset)	*Microcystis* sp., *Gleotrichia* sp.	–	No statistically significant health associations	No quantitative cyanobacterial cell densities provided
Philipp et al. (1992)	Cross-sectional n = 246 United Kingdom (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 HPLC with photodiode array detection or ELISA; cylindro-spermopsin 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]

Reference	Study Design, n, and Location	Cyanobacteria Identified	Cyanotoxins Measured	Health Association[a]	Lowest Significant Cyanobacterial Cell Density (cells/mL)
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
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-MS	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

sp. = unspecified species of the genus; HPLC = high performance liquid chromatography; MS = mass spectrometry; MS/MS = tandem mass spectroscopy

[a] Includes only significant associations between recreational cyanobacteria exposure and health effects.

[b] Values were converted from cyanobacterial cell surface area (> 12.0 mm^2/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 and 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 studies (El Saadi et al. 1995; Philipp 1992; Philipp and 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 five 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 seven 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 seven 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 percent 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 percent 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 percent 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 to 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 percent CI: 0.65–3.51; 20,000–100,000 cells/mL: RR = 2.71, 95 percent CI: 1.02–7.16; > 100,000 cells/mL: RR = 3.28, 95 percent 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 microcystin concentrations varied by lake and by sample location (littoral versus limnetic). Microcystins were 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 (first tertile: < 0.0012 μg/L; second tertile: 0.0012–0.2456 μg/L; third tertile: > 0.2456 μg/L). Symptoms were examined in relation to recreational and drinking water exposure to cyanobacteria and microcystins. Only GI symptoms were associated with recreational contact. The highest microcystin concentration at which an episode of GI symptoms was reported was 7.65 μg/L. There was no significant increase in adjusted RR of GI symptoms with recreational exposure to more than 1 μg/L microcystins. Adjusted RR (adjusted for gender, gastrointestinal (GI) 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 percent CI=0.32–3.52) and 1.48 (95 percent CI = 0.41–5.23), respectively. There were significant increases in adjusted RR 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 (e.g., 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 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 to 1461 cells/mL).

The Lin et al. (2015) 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, OR = 1.30, 95 percent CI: 1.08–1.56; ≥ 75th percentile, OR = 1.37, 95 percent 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 percent CI = 1.05–1.66) and earache (OR = 1.75, 95 percent 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 dermatoxins, debromoaplysiatoxin, and lyngbyatoxin, using HPLC-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 and 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 three percent of total planktonic cyanobacteria (other than picocyanobacteria). It is also possible that the cyanobacterial cells or associated contaminants 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. 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 and 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 microcystins at levels greater than 20 μg/L. GI 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, nine percent, and nine percent of cases reported, respectively).

During 2009 and 2010 in the United States, 11 outbreaks of illness associated with HABs were reported to CDC, all occurring in freshwater lakes and reported via the National Outbreak Reporting System (NORS) and the Harmful Algal Bloom-related Illness Surveillance System (HABISS). Hilborn et al. (2014) analyzed the HAB outbreak data from 2009–2010 and found the 11 outbreaks affected at least 61 persons, resulting in two hospitalizations, and included GI, dermatologic, respiratory, neurologic, and other symptoms. Sixty-six percent of case patients were individuals aged one to 19 years (n = 38 of 58 total) and 35 percent were aged nine years or younger (n = 20). In addition, in a cyanobacteria-associated outbreak in 2001, 42 children were affected. Outbreak data are typically limited in scope and thought to represent an underreporting of the "true" occurrence of illness in a population, but available

information suggests that children may share a disproportional share of the health burden associated with recreational exposures to cyanobacterial HABs.

Dziuban et al. (2006) and Walker et al. (2008) reported on outbreaks in Nebraska. Dziuban et al. (2006) described two 2004 cyanobacteria-associated outbreaks in which 22 cases of illness were reported from exposure to Nebraska lakes. The predominant illnesses in both outbreaks included dermatitis and gastroenteritis, and individuals who sought medical care showed a combination of rashes, diarrhea, cramps, nausea, vomiting, and fevers. Walker et al. (2008) also reported about a Nebraska outbreak. Levels of total microcystins at the east swimming beach of Pawnee Lake exceeded 15 ppb on July 12, 2004, and a health alert was issued. However, heavy public use of Pawnee Lake occurred that weekend and more than 50 calls were received from the public, complaining about symptoms such as skin rashes, lesions, blisters, vomiting, headaches, and diarrhea after swimming or water skiing in Pawnee Lake (Walker et al., 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 non-toxin-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 *Microcystis aeruginosa* strains. Serum from these individuals was collected from a subset of 15 patients who elicited strong skin test responses to *Microcystis aeruginosa* and from three healthy control subjects. The lysate from nontoxic *Microcystis 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 *Microcystis 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 *Microcystis aeruginosa* extracts using rat basophil leukemia cells. The authors concluded that the same allergen is present in toxic and nontoxic *Microcystis aeruginosa* lysates, but suggest the toxic *Microcystis 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 *Microcystis aeruginosa* lysate indicated that either linker core-membrane peptide or phycocyanin, or both, are potentially responsible for *Microcystis 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 LPS 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 LPS 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 LPS 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 LPS found that pre-exposure to LPS increased the lethal concentration (LC_{50}) of cylindrospermopsin eight-fold (Lindsay et al. 2006). The authors concluded that the decrease in susceptibility to cylindrospermopsin was due to the effects of LPS on detoxification enzyme pathways; LPS 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

This appendix describes in detail the approach used to derive the value for ingestion rate in units of liters per day. The ingestion rate is used in the derivation of the recommended cyanotoxin values in this document.

To arrive at liters of ingestion per day, the EPA combined data on liters of ingestion per hour and the number of hours spent in the water per day. Both of these parameters were represented as log-normal distributions. The sources of the data were:

- Recreational water ingestion per hour – The lead author of Dufour et al. (2017) provided the EPA's Office of Water, Health and Ecological Criteria Division with the raw data collected and analyzed in the study, which included mL of water ingested during a swimming event. Each participant in the study also reported the length of time they spent in the water. The ingestion per event was normalized to one hour for each participant and converted to liters to arrive at liters ingested per hour. The mean and standard deviation were calculated for different age groups (6 to 10, 11 to 17, 18 years and up, and all ages). See Table E-1 below for summary statistics for this parameter. Subsequent to the EPA's analysis, Dufour et al. posted their raw dataset on data.gov (U.S. EPA 2018). There are few minor variations in the dataset analyzed here and the posted dataset (i.e., the posted dataset included an additional adult participant's results, specified time spent in the water as 45 minutes for two participants, rounded ingestion volumes of 0.5 up to 1, and indicated a higher ingestion volume for one adult woman). The EPA performed a sensitivity analysis to see if these differences impacted the results and found no significant effect. The very slight differences were within the rounding to the third decimal. No differences were observed between the datasets for the results of the combined distribution analysis for the six- to 10-year age group.

- Duration of swimming per day – the EPA's 2011 *Exposure Factors Handbook* (EFH; Table 16-20). Time Spent (minutes/day) in Selected Outdoor Locations, Doers Only, At Home in the Outdoor Pool or Spa). Table E-2 below shows the summary statistics provided by the EPA's EFH.

Table E-1. Parameters Used to Fit Ingestion Distributions

Ingestion Rate (L/hour)				
Age Group (sample size)	**Mean[a]**	**Standard deviation**	**Minimum**	**Maximum**
6 to 10 (child) (n = 66)	0.03745	0.03355	0.00033	0.20000
11 to 17 (child) (n = 170)	0.03996	0.04377	0.00067	0.26800
18+ (adult) (n = 312)	0.02811	0.04960	0.00012	0.36800
All (6 to 50+) (n = 549)	0.03290	0.04643	0.00012	0.36800

[a] Arithmetic mean based on raw data provided by the Dufour et al. (2017) study authors. The ingestion rates for age groups children (6 to 10), teens (11 to 15), and adults (16 and over) were reported as geometric means in Dufour et al. (2017).

Table E-2. Parameters Used to Fit Recreation Duration Distributions

EPA 2011 EFH (Excerpt from Table 16-20) (minutes/day)					
Age Group (sample size)	**Mean**	**Standard deviation**	**Median (50th percentile)**	**Minimum**	**Maximum**
1 to 4 (n = 9)	85.6	86.3	60	15	255
5 to 11 (n = 15)	164.2	103.97	140	25	450
12 to 17 (n = 5)	97	53.8	100	40	180
18 to 64 (n = 44)	117.6	112.7	83	4	450
> 64 (n = 10)	78.9	85.3	53	1	258

R (open source programming language) was used to perform the calculations described in this appendix. The annotated R code is shown below, following a summary of what calculations were performed and assumptions.

The water ingestion rate per hour data from Dufour were used to compute an arithmetic mean and standard deviation, which are in turn used to compute the log geometric mean (GM) and log geometric standard deviation (GSD) using a mathematical conversion formula. The log GM and log GSD are used as distributional parameters to generate 10,000 random samples representing water ingestion rates per hour of recreational activity (L/hour).

The mean and standard deviation of the number of recreational hours spent in the water per day are reported as summary statistics in the EFH 2011, and are used to compute the log GM and log GSD using a mathematical conversion formula. The log GM and log GSD are used as distributional parameters to generate 10,000 random samples representing water ingestion rates per hour of recreational activity (hour/day).

The two component distributions are assumed to be statistically independent of each other and are multiplied to generate a combined distribution with 10,000 values for the ingestion rate of water per day of recreational activity in L/day. Summary statistics, including the mean, standard deviation, and point estimates of various percentiles, are then computed from the combined distribution. The EPA chose the 90th percentile point estimate for children six to 10 (0.21 L/day) to calculate the recommended cyanotoxin values.

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R Code

```
#Cyanotoxin recAWQC WA

# This script is to combine distributions for water ingestion rate (L/hr) and recreational exposure duration (hr/day) to develop a distribution for ingestion/day (L/day) and to generate a histogram of this combined distribution

# The first distribution is the incidental ingestion rate per hour from the Dufour dataset

# The second distribution is the recreational exposure duration (hr/day) from the EPA 2011 Exposure Factors Handbook Table 16-20. Time Spent (minutes/day) in Selected Outdoor Locations, Doers Only, At Home in the Outdoor Pool or Spa

# Both distributions are assumed to be log-normal


#####Read required libraries and set simulation sample size #################


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


library(truncnorm) #import library for truncated normal distribution


nsamp = 1000000 # specify number of samples in monte-carlo analysis


set.seed(1984756) # set seed for analysis replicability


#########################################################


# The combined distribution function (cdist) assumes a log-normal distribution for ingestion rate (L/hour) and a log-normal distribution for exposure duration (hr/d)

#....using the mean and sd as parameter inputs. This function is called in later sections of the code for each age group analysis.


cdist<-function(nsamp,mean_dur,sd_dur,min_dur,max_dur,mean_ing,sd_ing,min_ing,max_ing){
```

```
n<-nsamp # number of samples to be drawn

#transform mean and sd of duration

sd_dur_ln<-sqrt(log((sd_dur/mean_dur)^2+1)) # standard deviation of duration in log space
mean_dur_ln<-log(mean_dur)-((sd_dur_ln^2)/2) # mean of duration in log space
min_dur_ln<-log(min_dur) # minimum duration in log space
max_dur_ln<-log(max_dur)

#transform mean and sd 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)

# draw n samples from the truncated ingestion rate distribution in L/hr

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

# draw n samples from the truncated duration distribution (hr/d)

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

# compute n samples for the combined ingestion rate per day distribution (L/d)

ingperday<-ingperhr_ln_trunc*duration_hr_ln_trunc #combine distributions
```

```
  print(summary(ingperday)) # print summary statistics of the combined distribution

  print(quantile(ingperday, probs=0.90)) # print 90th percentile of the combined distribution

  #Generate histogram

  hist(ingperday,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Truncated hybrid distribution fit", xlim=c(0, 2.0), ylim=c(0, 1))
  h=hist(ingperday)
  h$density=h$counts/sum(h$counts)
  plot(h,xlab="Ingestion rate (L/day)",ylab="Probability", main ="Log-normal distribution fit", xlim=c(0, 1), ylim=c(0, 0.99), xaxp=c(0,1.5,15), freq=FALSE)

}

#########################################################

#I. Analysis for 6 to 10 age group

# These values are from 2011 EFH table 16-20 for ages 5 to 11.

mean_dur_min=164.2
sd_dur_min=103.97
min_dur_min=25
max_dur_min=450

# Convert exposure data from the EPA's EFH from min/day to hr/day

mean_dur<-mean_dur_min/60 #mean exposure duration hr/day
```

```
sd_dur<-sd_dur_min/60 #sd 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


# These ingestion rate values are computed from the Dufour dataset


mean_ing<- 0.03745 # mean ingestion rate in L/hr

sd_ing<-0.03355 # sd ingestion rate in L/hr

min_ing<-0.00033 # minimum ingestion rate in L/hr

max_ing<-0.20000 # maximum ingestion rate in L/hr


cdist(nsamp,mean_dur,sd_dur,min_dur,max_dur,mean_ing,sd_ing,min_ing,max_ing) # call combined
distribution function



#######################################################


#II. Analysis for 11 to 17 age group


# These values are from 2011 EFH table 16-20 for age 12 to 17

mean_dur_min=97

sd_dur_min=53.81

med_dur_min=100

min_dur_min=40

max_dur_min=180


# Convert exposure data from the EPA's EFH from min/day to hr/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


# These ingestion rate values are computed from the Dufour dataset


mean_ing<-0.03996 # mean ingestion rate in L/hr

sd_ing<-0.04377 # sd ingestion rate in L/hr

min_ing<-0.00067 # minimum ingestion rate in L/hr

max_ing<-0.26800 # maximum ingestion rate in L/hr


cdist(nsamp,mean_dur,sd_dur,min_dur,max_dur,mean_ing,sd_ing,min_ing,max_ing) # call combined
distribution function



#####################################################


#III. Analysis for 18+ age group

# Combine exposure duration data for 18 to 64 and for >64 age groups from 2011 EFH table 16-20.


mean_dur_min=(117.61+78.9)/2

sd_dur_min=sqrt((112.72^2+85.32^2)/2)

min_dur_min=1

max_dur_min=450


# Convert exposure data from the EPA's EFH from min/day to hr/day


mean_dur<-mean_dur_min/60 #mean exposure duration hr/day
```

```
sd_dur<-sd_dur_min/60 #sd 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


# These ingestion rate values are computed from the Dufour dataset


mean_ing<-0.02811 # mean ingestion rate in L/hr

sd_ing<-0.04960 # sd ingestion rate in L/hr

min_ing<-0.00012 # minimum ingestion rate in L/hr

max_ing<-0.36800 # maximum ingestion rate in L/hr


cdist(nsamp,mean_dur,sd_dur,min_dur,max_dur,mean_ing,sd_ing,min_ing,max_ing) # call combined
distribution function



#########################################################


# IV. Analysis for all age groups (including 1-4 yo)


# Combine exposure duration data for all age groups (1 to 4, 5 to 11, 12 to 17, 18 to 64, >64) from 2011
EFH table 16-20.


mean_dur_min=(85.56+164.2+97+117.61+78.9)/5

sd_dur_min=103.71 # SD reported in EFH for all ages

min_dur_min=1

max_dur_min=450


# Convert exposure duration data from min/day to hr/day


mean_dur<-mean_dur_min/60 #mean exposure duration hr/day
```

sd_dur<-sd_dur_min/60 #sd 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

These ingestion rate values are computed from the Dufour dataset

mean_ing<- 0.03290 # mean ingestion rate in L/hr

sd_ing<- 0.04643 # sd ingestion rate in L/hr

min_ing<-0.00012 # minimum ingestion rate in L/hr

max_ing<-0.36800 # maximum ingestion rate in L/hr

cdist(nsamp,mean_dur,sd_dur,min_dur,max_dur,mean_ing,sd_ing,min_ing,max_ing) # call combined distribution function

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APPENDIX F. INGESTION STUDIES

The EPA reviewed seven studies to evaluate recreation-associated incidental ingestion (DeFlorio-Barker et al. 2017; Dorevitch et al. 2011; Dufour et al. 2006, 2017; Schets et al. 2011; Schijven and de Roda Husman 2006; Suppes et al. 2014). Evans et al. 2006 was also reviewed, but is the same dataset as Dufour et al. (2017), so it is not included in the table. The EPA's approach for estimating incidental exposure while swimming used by the EPA's Office of Pesticide Programs (OPP) is also summarized below.

F.1 DeFlorio-Barker et al. (2017)

DeFlorio-Barker et al. (2017) combined ingestion data from Dufour et al. (2017) and time spent in the water data from 12 cohorts of epidemiological studies to estimate the volume of water ingested per swimming event. They calculated the ingested volume per minute (mL/minute) for each Dufour et al. (2017) study participant, using the mL ingested and the self-reported time spent in the water for each participant. The National Epidemiological and Environmental Assessment of Recreational Water Study and Southern California Coastal Water Research Project epidemiological studies included 68,685 recreators at four freshwater and eight marine beaches. The participants in these studies estimated how much time they spent in the water. DeFlorio-Barker et al. (2017) combined the mL/minute ingestion rate from Dufour et al. (2017) and the self-reported time spent in the water for the epidemiological study participants to calculate the volume of water ingested per event. The results of this study corroborate other studies that demonstrate that, on average, children have higher incidental ingestion than adults when recreating.

F.2 Dorevitch et al. (2011)

Dorevitch et al. (2011) evaluated incidental ingestion associated with multiple types of water contact activities in both surface water (canoeing, fishing, kayaking, motor boating, and rowing) and in pools (canoeing, fishing, kayaking, swimming, wading/splashing, and walking around the pool as a control). The surface water activities did not include swimming because the water body was designated for secondary contact recreation only. Volume of ingestion was self-reported via interviews (3,367 participants: 2,705 individuals recreating in the Chicago Area Waterway System (CAWS, surface water) and 662 individuals recreating at a public outdoor swimming pool). At the end of their exposure, participants self-reported whether they ingested water, and how much, during their recreational experience. 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).

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 95 percent upper confidence limit ingestion volumes between 0.01 and 0.012 L/hour. Less than five percent of limited contact recreators on surface waters reported swallowing any water. The study authors considered those who capsized during canoeing or kayaking a "middle ingestion category," with mean incidental ingestions of 0.006 to 0.005 L/hour. Swimmers were the highest ingestion category, with a mean of 0.01 L/hour. 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.

In surface water, participants ages six years 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). Kayaking and capsizing in surface water resulted in nearly as high incidental ingestion (mean = 0.005 L; Upper 95 percent CI: 0.0165). In swimming pool water, participants ages six 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). Duration of activities was not reported, so the ingestion volumes are on a per event basis.

F.3 Dufour et al. (2006)

The EPA's *Exposure Factors Handbook* (EFH) (2011) presents values for incidental ingestion while recreating values citing Dufour et al. (2006). 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 2006 study design instructed participants to swim for at least 45 minutes, so the time the participants spent in the water is probably not representative of preferred or regular patterns for recreation duration and the actual duration was not recorded. Both studies reported higher ingestion among children compared to adults. The values presented in the EFH adjusted the Dufour et al. (2006) data from a per event basis to an hourly ingestion rate. The EFH 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).

F.4 Evans et al. (2006)

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. This study characterized ingestion volumes for younger children verses older children and adults. Evans et al. (2006) reported higher ingestion volumes for younger children. Although study results were presented at a conference, they were not published, so the EPA did not cite this publication in the derivation of the recommended cyanotoxin values. However, Dufour et al. (2017) includes the data reported by Evans et al. (2006).

F.5 Schets et al. (2011)

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. Of the 8,000 adults who completed the questionnaire, 1,924 also provided estimates for their eldest child (< 15 years of age). The participants estimated the amount of water they or their children swallowed while swimming. Participants chose between four categories of water volumes: (1) no water or only a few drops; (2) one to two mouthfuls (a shot glass); (3) three to five mouthfuls (coffee cup); and (4) six to eight mouthfuls (soda glass). 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 per event. Adult men swallowed, on average 0.030 L/hour and women swallowed 0.020 L/hour, with somewhat greater ingestion in marine waters than in freshwater or a swimming pool. In fresh and marine waters children swallowed about the same as adults, and in swimming pools they ingested more than adults, on average, 0.038 L/hour compared with 0.030 and 0.021 for males and females, respectively (Schets et al. 2011). The EPA made the assumption that

exposure in a swimming pool is roughly equivalent to exposure in fresh and marine waters. Schets (2011) supports that assumption, although it is a somewhat more conservative assumption for children. However, when bodyweight is taken into account the greater exposure to children versus adults becomes clear. Additional research would be helpful to clarify uncertainty in differences in ingestion from different types of waters.

F.6 Schijven and de Roda Husman (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 mean ingestion rates in freshwater ranged from 0.0015 to 0.019 L/hour, with the highest mean being for adult recreational divers wearing an ordinary diving mask and the lowest mean for adult recreational divers wearing a full face mask. The mean ingestion rates in marine water ranged from 0.0005 to 0.014 L/hour, with the highest mean being for adult recreational divers wearing an ordinary diving mask and the lowest mean for adult recreational divers wearing a full face mask. The age of the divers was not included in the study report. Occupational divers dived on average 60–95 minutes and sport divers dived on average 42–52 minutes per dive.

F.7 Suppes et al. (2014)

Suppes et al. (2014) used a similar measurement method as Dufour et al. (2006, 2017), (i.e., using cyanuric acid as an indicator of pool water ingestion) to evaluate the rate of water ingested by 16 children ages five to 17 years. They 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/hour with range 0–0.051 L. The mean rate at which children ingested water was 0.026 L/hour with range 0.0009–0.106 L/hour.

F.8 U.S. EPA (2003)

Additional estimates of incidental water ingestion rates while swimming in pools have been identified by the EPA's OPP. OPP calculated people's exposures to pool chemicals while they swim using its Swimmers Exposure Assessment Model (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/hour and 0.025 L/hour, respectively. The model assumes an incidental ingestion rate of 0.050 L/hour for children ages seven to 10 years and 11 to 14 years while swimming noncompetitively. The 0.050 L/hour value is the value used

in the EPA OPP's Standard Operating Procedures (U.S. EPA 2000) and is based on recommendations from EPA's Risk Assessment Guidance for Superfund, Part A (U.S. EPA 1989, 2000, 2003).

F.9 Summary

Although these studies used different methodologies and have limitations with respect to reporting information for different age group categories, their results show a similar pattern compared to Dufour et al. (2006, 2017): children ingest water at a higher rate while swimming than adults. Dufour et al. (2017) and Dufour et al. (2006) identified mean ingestion rates for children of 0.037–0.040 and 0.049 L/hour, respectively, and adult rates of 0.028 and 0.021 L/hour, respectively. Depending on water type, Schets et al. (2011) found a mean ingestion volume for children aged zero to 14 years of 0.028–0.038L/hour for children and 0.020–0.036 L/hr for males and females. The most pronounced differences were for swimming pools, where children ingested at a higher rate (0.038 L/hour) than adults (males: 0.030 L/hour; females: 0.021 L/hour). Dorevitch et al. (2011) reported ingestion rates while swimming for all ages of 0.010L/hour. Suppes et al. (2014) reported an adjusted mean ingestion rate of 0.026 L/hour for children and a rate of 0.0035 L/hour for adults.

Table F-1 includes: sample size, measurement methodology, the maximum values or the upper confidence intervals (CI) for the mean ingestion per event, time spent in the water (mean or range), and the mean ingestion volume normalized to one hour (or range if a range of durations were reported). This information supports comparison of the studies and help with understanding the range of different recreational exposures from activities.

The column with normalized ingestion (mL/hour) was populated using the following methods:

- Dufour et al. (2017) – The EPA used the individual data points from this dataset. Each participant's volume ingested was adjusted to one hour based on the length of time that participant reported being in the water.

- Dufour et al. (2006) – The EPA assumed that all swimming events were 45 minutes in duration. The values reported in Table F-1 are the same as the values in EPA's EFH (2011).

- DeFlorio-Barker et al. (2017) – Normalized data are not included in Table F-1 because the authors used the Dufour et al. (2017) rate in their modeling, so including the normalized data would be duplicative of Dufour et al. (2017).

- Dorevitch et al. (2011) – Study authors included normalized values in the study publication.

- Schets et al. (2011) – The EPA used the mean duration values provided in the publication to calculate the normalized value for each age and activity category.

- Suppes et al. (2014) – Study authors reported volume per hour.

- Schijven and de Roda Husman (2006) – The EPA used the range of duration values provided in the publication to calculate the normalized value for each activity category.

F.10 References

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Table F-1. Studies of Incidental Ingestion Volumes While Recreating

Reference	Study Sample Size	Measurement Methodology	Water Type, Recreational Activity	Age Group[a] (Years Old)	Mean Ingestion (Maximum Value) (mL/event)	Mean Duration of Event (minutes)	Normalized Ingestion (mL/hour)
Dataset from Dufour (data collection methods reported in Dufour et al. (2017))[b]	>500	Cyanuric acid was measured in pool water and urine samples, and ingestion rate was calculated based on duration of swimming event	Swimming pool, Swimming	6 to 10	59.8 (245)	each participant reported a duration[c]	37
				11 to 17	35.6 (267)	each participant reported a duration[c]	40
				18+	23.7 (279)	each participant reported a duration[c]	28
				All ages (6+)	31.7 (279)	each participant reported a duration[c]	33
Dufour et al. (2006)	53	Cyanuric acid was measured in pool water and urine samples	Swimming pool, Swimming	6 to ≤ 18	37 (NR)	≥ 45	49
				18+	16 (NR)	≥ 45	21
				All ages (6+)	32 (NR)	≥ 45	43
DeFlorio-Barker et al. (2017)	12 cohorts totaling 68,685	Estimates of amount of water swallowed were self-reported	Freshwater	6 to 10	58.9 (142)[d]	(NR)	–
				11 to 17	55.5 (140) [d]	(NR)	–
				18+	21.9 (46.7)[d]	(NR)	–
			Marine Water	6 to 10	74.4 (180)[d]	(NR)	–
				11 to 17	75.6 (186.7)[d]	(NR)	–
				18+	32.4 (72) [d]	(NR)	–
Dorevitch et al. (2011)	3,367	Estimates of amount of water swallowed were self-reported	Surface water, Canoeing/capsizing	All ages (6+)	6 (19.9)[e]	No duration constraints	–
			Surface water, Kayaking/capsizing	All ages (6+)	5 (16.5)[e]	No duration constraints	–
		Estimates of amount of water swallowed were self-reported; cyanuric acid was measured in urine in a subset of participants	Swimming pool, Swimming	All ages (6+)	10 (34.8)[e]	60	10
			Swimming pool, Canoeing/capsizing	All ages (6+)	6.6 (22.4)[e]	60	6.6
			Swimming pool, Kayaking/capsizing	All ages (6+)	7.9 (7.9)[e]	60	7.9

Reference	Study Sample Size	Measurement Methodology	Water Type, Recreational Activity	Age Group[a] (Years Old)	Mean Ingestion (Maximum Value) (mL/event)	Mean Duration of Event (minutes)	Normalized Ingestion (mL/hour)
Schets et al. (2011)	9,924 (1,924 of which were children)	Descriptive estimates of the amount of water swallowed were self-reported by participants or parents of participants, and estimates were converted to volumes	Freshwater, Swimming	0 to 14	37 (170)[e]	79	28
				15+, males	27 (140)[e]	54	30
				15+, females	18 (86)[e]	54	20
			Marine water, Swimming	0 to 14	31 (140)[e]	65	29
				15+, males	27 (140)[e]	45	36
				15+, females	18 (90)[e]	41	26
			Swimming pool, Swimming	0 to 14	51 (200)[e]	81	38
				15+, males	34 (170)[e]	68	30
				15+, females	23 (110)[e]	67	21
Suppes et al. (2014)	38	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	Swimming pool, Swimming (adjusted)	5 to 17	26 (106)	60[f]	26
				18+	4 (51)	60[f]	3.5
				All ages (5+)	14 (106)	60[f]	14
			Swimming pool, Swimming (unadjusted)	5-17	59 (225)	60[f]	59
				18+	9 (NR)	60[f]	9
				All ages (5+)	32 (NR)	60[f]	32
Schijven and de Roda Husman (2006)	517	Descriptive estimates of the amount of water swallowed were self-reported, and estimates were converted to volumes	Freshwater, Recreational diving w/ordinary diving mask	Adults	13 (190)	42 to 52	15 to 19
			Freshwater, Recreational diving w/full face mask	Adults	1.3 (15)	42 to 52	1.5 to 1.9
			Freshwater, Occupational diving	Adults	5.7 (25)	60 to 95	4 to 6
			Marine Water (coastal), Recreational diving w/ordinary diving mask	Adults	9.9 (190)	42 to 52	11 to 14

Reference	Study Sample Size	Measurement Methodology	Water Type, Recreational Activity	Age Group[a] (Years Old)	Mean Ingestion (Maximum Value) (mL/event)	Mean Duration of Event (minutes)	Normalized Ingestion (mL/hour)
			Marine water (coastal), Recreational diving w/full face mask	Adults	1.3 (15)	42 to 52	1.5 to 1.9
			Marine Water (open sea), Recreational diving w/ordinary diving mask	Adults	7.7 (100)	42 to 52	9 to 11
			Marine water (open sea), Recreational diving w/full face mask	Adults	0.43 (2.8)	42 to 52	0.5 to 0.6
			Marine Water (coastal and open sea combined), Recreational diving w/ordinary diving mask	Adults	9.0 (190)	42 to 52	10 to 13
			Marine water (coastal and open sea combined), Occupational diving	Adults	9.8 (100)	60 to 95	6 to 10
			Swimming pool, Recreational diving w/ordinary diving mask	Adults	20 (190)	42 to 52	23 to 29
			Swimming pool, Recreational diving w/full face mask	Adults	13 (190)	42 to 52	15 to 19

[a] Age group ranges reflect the age groupings reported in the study. In some cases the authors did not separate data by different age groups among children or between adults and children.

[b] The values shown are arithmetic means calculated from the Dufour dataset. The Dufour et al. (2017) publication reported ingestion volumes as geometric means for children (6 to 10 years), teens (11 to 15 years), and adults (16 years and over).

[c] Each participant's volume ingested was adjusted to one hour based on the length of time that participant reported being in the water.

[d] No maximum values are reported in the study; 90th provided percentile in parentheses.

[e] No maximum values are reported in the study; upper limit of the CI is provided.

[f] Swimming duration was reported as ≥ 45 minutes, however authors derived and reported only hourly ingestion per event.

APPENDIX G. INFORMATION ON CELLULAR CYANOTOXIN AMOUNTS

The information in the tables in this appendix 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.

The information in Tables G-3 and G-4 was generated from both a brief survey and a standardized search of the peer-reviewed and published scientific literature. The purpose of these searches was to evaluate the availability of cyanotoxin quota data (i.e., cyanotoxin content per cyanobacterial cell or per unit biomass, for microcystins and cylindrospermopsin) needed to calculate a cyanobacterial cell density potentially associated with a specific cyanotoxin concentration.

The EPA conducted a brief initial survey of the available peer-reviewed and published scientific literature in December 2016 and identified 29 studies with data on cellular toxin amounts. After reviewing the available data, a formal literature search was conducted. The purpose of this literature search and screening was to identify literature relevant to answering the following research question: What cyanotoxin cell quota data (i.e., cyanotoxin content per cyanobacterial cell or per unit biomass, for microcystins and cylindrospermopsin) are available in the peer-reviewed literature?

Search terms were identified with support from a subject matter expert and library science professionals and included genera of known microcystins or cylindrospermopsin producers, names of the toxins of interest, and keywords that could indicate that quota data were reported. The search was conducted in PubMed and results were limited to articles published in English from 1987 to March 2017. A summary of the literature search results is provided in Table G-1.

Table G-1. Summary of Cyanotoxin Cell Quota Data Literature Search Results

Database	Results	Notes/Limits
PubMed	253	1987 to present; English
Web of Science	472	1987 to present; English
Total Unique	**485**	

The EPA developed search strategies for each database. Both search strategies included the same set of keywords but varied in how these keywords were strung together. The Web of Science search strategy also included limits, a feature not characteristic of a search strategy conducted using PubMed. The search strategies are provided below.

PubMed

Date of Search: 3/01/2017
Date Limit: 1987 to present
Language = English

Set	PubMed Search Strategy
1	(Anabaena[tiab] OR Anabaena[mh] OR Anabaena-flos-aquae[tiab] OR Anabaenopsis[tiab] OR Aphanizomenon[tiab] OR Aphanizomenon[mh] OR C.-raciborskii[tiab] OR Chrysosporum-ovalisporum[tiab] OR Cuspidothrix[tiab] OR Cylindrospermopsis[tiab] OR Cylindrospermopsis[mh] OR Cylindrospermopsis-raciborskii[tiab] OR Cylindrospermum[tiab] OR Dolichospermum[tiab] OR Fischerella[tiab] OR Gloeotrichia[tiab] OR Lyngbya[tiab] OR

Set	PubMed Search Strategy
	M.-aeruginosa[tiab] OR Microcystis[tiab] OR Microcystis[mh] OR Microcystis-aeruginosa[tiab] OR Nostoc[tiab] OR Nostoc[mh] OR Oscillatoria[tiab] OR Oscillatoria[mh] OR Phormidium[tiab] OR Planktothrix[tiab] OR Sphaerospermopsis[tiab] OR Synechococcus[tiab] OR Synechococcus[mh])
2	AND (microcystin[tiab] OR microcystins[tiab] OR microcystins[mh] OR cylindrospermopsin[tiab] OR cylindrospermopsin[Supplementary Concept])
3	AND (quota[tiab] OR cell-content[tiab] OR cellular-concentration[tiab] OR cyanotoxin-content[tiab] OR intracellular-content[tiab] OR intracellular-concentration[tiab] OR toxin-content[tiab] OR microcystin-content[tiab] OR microcystin-LR-content[tiab] OR MC-content[tiab] OR MCYST-content[tiab] OR MC-LR-content[tiab] OR intracellular-microcystin[tiab] OR intracellular-MC[tiab] OR microcystin-production[tiab] OR microcystin-LR-production[tiab] OR microcystins-production[tiab] OR MC-production[tiab] OR MCYST-production[tiab] OR MC-LR-production[tiab] OR CYN-content[tiab] OR particulate-CYN[tiab] OR cylindrospermopsin-production[tiab])

Web of Science
Date of Search: 3/01/2017
Date Limit: 1987 to present
Language = English
All terms searched in Topic (Title, Abstract, and Keywords)

Set	Web of Science Search Strategy
1	(Anabaena OR Anabaena-flos-aquae OR Anabaenopsis OR Aphanizomenon OR C.-raciborskii OR Chrysosporum-ovalisporum OR Cuspidothrix OR Cylindrospermopsis OR Cylindrospermopsis-raciborskii OR Cylindrospermum OR Dolichospermum OR Fischerella OR Gloeotrichia OR Lyngbya OR M.-aeruginosa OR Microcystis OR Microcystis-aeruginosa OR Nostoc OR Oscillatoria OR Phormidium OR Planktothrix OR Sphaerospermopsis OR Synechococcus)
2	AND (microcystin OR microcystins OR cylindrospermopsin)
3	AND (microcystin-RR-content OR MC-RR-content OR particulate-microcystin OR particulate-MC OR cylindrospermopsin-content OR intracellular-CYN OR quota OR cell-content OR cellular-concentration OR cyanotoxin-content OR intracellular-content OR intracellular-concentration OR toxin-content OR microcystin-content OR microcystin-LR-content OR MC-content OR MCYST-content OR MC-LR-content OR intracellular-microcystin OR intracellular-MC OR microcystin-production OR microcystin-LR-production OR microcystins-production OR MC-production OR MCYST-production OR MC-LR-production OR CYN-content OR particulate-CYN OR cylindrospermopsin-production)
Limits	AND Research Areas: (AGRICULTURE OR OCEANOGRAPHY OR ENVIRONMENTAL SCIENCES ECOLOGY OR PHARMACOLOGY PHARMACY OR EVOLUTIONARY BIOLOGY OR BIOCHEMISTRY MOLECULAR BIOLOGY OR FISHERIES OR PLANT SCIENCES OR BIODIVERSITY CONSERVATION OR PUBLIC ENVIRONMENTAL OCCUPATIONAL HEALTH OR RESEARCH EXPERIMENTAL MEDICINE OR BIOTECHNOLOGY APPLIED MICROBIOLOGY OR SCIENCE TECHNOLOGY OTHER TOPICS OR CELL BIOLOGY OR CHEMISTRY OR LIFE SCIENCES BIOMEDICINE OTHER TOPICS OR TOXICOLOGY OR MARINE FRESHWATER BIOLOGY OR WATER RESOURCES OR METEOROLOGY ATMOSPHERIC SCIENCES OR ZOOLOGY OR MICROBIOLOGY)

The EPA conducted title and abstract screening of the 253 search results (generated from both database searches) and classified them as "relevant," "maybe relevant," or "not relevant." Titles were considered "relevant" if the title or abstract included mention of cell quota data for microcystins or

cylindrospermopsin or if the title or abstract indicated that the study had quantitative information on cyanobacterial cell density and microcystins or cylindrospermopsin concentration and therefore may contain sufficient data to calculate a quota. Titles were considered "maybe relevant" if the title or abstract indicated the article might have information relevant to the research question. Title and abstract did not specifically include the term "quota" but indicated that it may have had quantitative information on cyanobacterial cell density and microcystins or cylindrospermopsin concentration or if cyanobacterial cells were only quantified by molecular methods such as PCR and toxin concentrations were measured. Titles were considered "not relevant" if the title/abstract did not appear to have information about microcystins or cylindrospermopsin quotas or densities/concentrations, if the study was a spiked cyanotoxin experiment (meaning cyanotoxins were added, not produced by cyanobacteria present), or if the study was not a peer-reviewed article, book, or government document.

The EPA prioritized the studies to facilitate the review. Prioritization yielded a high number of studies classified as "relevant" or "maybe relevant." Relevant studies were further prioritized for each cyanotoxin of interest based on date of publication. The approach for prioritization is presented in Table G-2. A full text review was conducted on Priority 1 studies only.

Table G-2. Summary of Study Prioritization

Toxin	Priority 1 Classification Criteria	Priority 2 Classification Criteria	Priority 3 Classification Criteria
Microcystins	Classified as relevant based on title/abstract screening; Did not use PCR quantification or evaluate benthic cyanobacteria; Identified predominant species without statistical analysis; Published in last 5 years; and Field study or study with both field and laboratory component.	Studies that use only PCR for quantification of cyanobacteria; and All laboratory studies (internal or external forcing, mitigation studies, studies evaluating non-nutrient pollutants).	Methods studies; and Studies on benthic cyanobacteria.
Cylindrospermopsin	Classified as relevant based on title/abstract screening; Did not use PCR quantification or evaluate benthic cyanobacteria; Identified predominant species without statistical analysis; Published in last 10 years; and Field study or laboratory study.	Studies that use only PCR for quantification of cyanobacteria.	Methods studies; and Studies on benthic cyanobacteria.

Extracted data from studies meeting the criteria for "Priority 1" are presented below in Table G-3 and are further summarized in Table G-4. Relevant quota data were extracted from both the text and figures in "Priority 1" studies. All figures were digitized using GraphPad Digitizer software, as appropriate. All extracted data from text and figures underwent primary and secondary review for quality assurance purposes.

The EPA's primary interest when reviewing the data was to identify the amount of toxin per cyanobacterial cell when toxin was present in a sample. In the environment, it is possible for cyanobacterial cells to be present with no toxin being produced (e.g., the cyanobacteria are a non-toxin-producing strain or environmental conditions do not support toxin production). The EPA only included quota data where toxin was detected.

The studies included in Table G-3 vary in methods used, conditions evaluated, and presentation of data. Typically, complete, raw data were unavailable. The EPA made choices regarding selection, presentation, aggregation, and conversion of data to develop the necessary standardization required for comparing and analyzing these data. Specifically, if quota values were from the same sample at a single location, the average and range were recorded; results from different sampling locations were recorded separately; and multiple mean quota values within the same study were recorded separately (note that separate mean values could be reported for different sampling sites or species within the same genera).

The EPA found that study authors report toxin quota data in various forms, including but not limited to toxin mass per cyanobacterial cell, toxin mass per cyanobacterial biomass, and toxin mass per cyanobacterial biovolume. Scientific measurement units vary among studies. The EPA presents the cell quota data in Table G-3 in the units reported by the study authors (i.e., without conversion to standard units). However, when possible, the EPA converted data to a standard set of units, picograms (pg) per cell, in Table G-4 so that data could be summarized and compared. The EPA did not identify appropriate conversion factors that would allow genus-specific conversion of quotas described in mass per biovolume to mass per cell or mass per biomass to mass per cell. The EPA considered converting biovolume quotas using methods cited in the Australian national guidelines (Australian Government National Health and Medical Research Council, 2008) and Ackaalan (2006), but ultimately decided that the number of uncertainties associated with these methods were too great. Thus, data with unique units are summarized separately in Table G-3, Table G-4, and Table 7-14.

Within Table G-3 and Table G-4, the EPA categorized studies as either "field" or "lab." Field studies include studies where environmental samples were collected and analyzed for cell quota data without additional manipulation of growing conditions. In some studies, environmental samples were taken to a laboratory where growing conditions were optimized or manipulated to determine cyanotoxin cell quota. These studies were categorized as laboratory studies. Other laboratory studies analyzed cell quota in laboratory strains that were not collected in the environment for the purpose of the analysis. For laboratory studies, only control data were extracted. In laboratory studies where there was no true control the conditions closest to ambient conditions were selected (e.g., multiple conditions were tested and none was the clear control, all data were included).

While the traditional definition of toxin quota refers to the intracellular amount of toxin, some studies presented the total toxin present normalized by the cell density or the extracellular toxin normalized by cell density as a quota. In other cases, methods for calculation of the quota were not very clear. If a quota value was presented (i.e., intracellular toxin per cell) this was recorded. If this value was not available or was not clearly described, was recorded as presented by the study authors and assumed to be intracellular or the total amount of toxin per cell. Extracellular toxin per cell was not recorded. The EPA recognizes that the exclusion of extracellular toxin data could lead to an underestimation of the amount of toxin per cell, in particular for cylindrospermopsin as *Cylindrospermopsis* has been shown to constitutively produce the toxin, which can stay inside the cells during log phase growth and accumulate externally upon entering the stationary phase (Davis et al. 2014; Burford et al. 2016). Researchers have also demonstrated that cylindrospermopsin production can be excreted in response to phosphorus limitation and induce other cells to excrete alkaline phosphatase to the water body resulting in a phosphorus scavenging effect (Bar-Yosef et al. 2010).

Some field studies identified the presence of cyanotoxins and multiple cyanobacterial genera including more than one potential toxin producer with no clear predominant toxin-producing species. Table G-3 only includes cell quota values from field studies where there was a clear predominant toxin-producing genera. In these instances, the study was grouped with the predominant toxin-producing genera. In mixed samples with multiple cyanobacteria and no predominant toxin-producing species, quota data were not included. The EPA recognizes that this approach presents a possible limitation to conclusions on toxin quota as studies conducted under non-bloom conditions were excluded. Predominant species are easier to identify when there is a bloom, however, traditional microscopic identification of cyanobacteria does not distinguish between toxigenic and non-toxigenic strains. The proportion of toxigenic cells within a cyanobacterial community and the copy number of the *mcyD* gene per cell can vary significantly, both affected by environmental parameters (Davis et al. 2009).

Table G-3 includes cell quota data for microcystin and cylindrospermopsin-producing genera. For each study, data are provided, where available, on the genus and species of the cyanobacteria, the site where the sample was collected or the clone used to estimate cellular toxin for, the type of study (i.e., field or laboratory), and the reported toxin quota data. Notes relevant to each study are reported in the final column of the table, when appropriate.

Relevant toxin data include the mean toxin quota per cell, the median toxin quota per cell, the minimum toxin quota per cell, or the maximum toxin quota per cell. These data are reported where available and not all data points were reported in each study. Data are presented using the units of measure reported by the study authors.

Table G-3. Cell Quota Data for Microcystin and Cylindrospermopsin-Producing Genera

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
Microcystin	*Microcystis* spp.	Grangent Reservoir, France	Field	Mean: 0.576 pg/cell Min: 0.042 pg/cell Max: 4.19 pg/cell	Sabart et al. (2013)	Data digitized from Figure 6b; The authors report cell quotas for different size ranges of *Microcystis aeruginosa* cells and these values represent the minimum and maximum for all sizes; Mean calculated using all cell quota data reported at all time points for all sizes; Study provides highest reported mean for *Microcystis* spp. mass per cell, field and field and lab combined
	Microcystis spp.	Lake Victoria, Kenya	Field	Mean: 17 fg/cell Median: 553 fg/cell	Sitoki et al. (2012)	Sixteen *Microcystis* strains identified
	Microcystis spp.	Lake Taihu, China	Field	Mean: 0.015 pg/cell Min: 0 pg/cell Max: 0.159 pg/cell	Wang et al. (2013)	Data digitized from Figure 4a,b; Mean calculated using all cell quota data reported at all time points for all colony sizes; Study provides minimum cell quota value and lowest reported mean for *Microcystis* spp. mass per cell, field, and field and lab combined
	Microcystis spp.	Dapugang River, Lake Taihu, China	Field	Cell quota data not presented	Xue et al. (2016)	
	Microcystis spp.	Umia River, Galicia, Spain	Field	Max: 570 µg/g biomass	Alvarez et al. (2016)	Mixed bloom: *Microcystis aeruginosa*, *Scenedesmus* spp., *Kirchneriella* spp.; unclear which is predominant
	Microcystis spp.	Lake Taihu, China	Field	Mean: 640.59 µg/g biomass	Wei et al. (2016)	Data digitized from Figure 4a,b; Only microcystin-L-R

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
				Min: 13.21 µg/g biomass Max: 1389.13 µg/g biomass		congener reported; Mean calculated using cell quota data for all time points; Study provides mean, minimum, and maximum cell quota values for *Microcystis* spp. mass per biomass, field
	Microcystis spp.	FACHB-905	Lab	Mean: 20.25 fg/cell Min: 17.05 fg/cell Max: 28.47 fg/cell	Wei et al. (2016)	Data digitized from Figure 1D and Figure 2D; Mean calculated using cell quota data for all time points
	Microcystis aeruginosa	Lake Huron, United States	Field	Mean: 140 fg/cell Mix: 10 fg/cell Max: 350 fg/cell	Fahnenstiel et al. (2008)	Study provides highest reported mean, maximum, and minimum cell quota values for *Microcystis aeruginosa* mass per cell, field, and field and lab combined
	Microcystis aeruginosa	Aguieira reservoir, Portugal	Field	Mean: 0.12 fg/cell Mix: 0.07 fg/cell Max: 0.22 fg/cell	Vasconcelos et al. (2011)	Data digitized from Figure 5; *Microcystis aeruginosa* was dominant microcystins producer; Mean calculated using all cell quota data for all yearly time points
	Microcystis aeruginosa	Lake Erie, United States	Field	Mean: 3.34 µg/mg biomass Min: 1.37 µg/mg biomass	Horst et al. (2014)	Data digitized from Figure 3 and Figure 6; Study provides mean and maximum cell quota value for *Microcystis aeruginosa* mass per biomass, field
	Microcystis aeruginosa	Hartbeespoort Dam, South Africa	Field	Min: 0.14 µg/g biomass Max: 268 µg/g biomass	Mbukwa and Mamba (2012)	Study provides minimum cell quota value for *Microcystis aeruginosa* mass per biomass, field
	Microcystis aeruginosa	BCCUSP232	Lab	Mean: 18.84 fg/cell Min: 15.07 fg/cell	Chia et al. (2016)	Data digitized from Figure 4b; Study provides lowest reported mean for

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
				Max: 22.61 fg/cell		*Microcystis aeruginosa* mass per cell, lab, field and lab combined and the minimum cell quota value for mass per cell, lab
	Microcystis aeruginosa	Model was used to simulate cyanobacteria	Lab	Mean: 91.5 fg/cell	Jähnichen et al. (2001)	Model used cell quota data reported by Long et al. (2001), Orr and Jones (1998), Jahnichen et al. (2001), and Watanabe et al. (1989); Study provides highest reported mean for *Microcystis aeruginosa* mass per cell, lab
	Microcystis aeruginosa	Model was used to simulate cyanobacteria	Lab	Min: 18 fg/cell Max: 23.7 fg/cell	Jähnichen et al. (2007)	Microcystins cell quota data reported in the presence of sodium and potassium, respectively; Study provides minimum cell quota value for *Microcystis aeruginosa* mass per cell, lab
	Microcystis aeruginosa	MASH01 non-axenic	Lab	Mean: 84.7 fg/cell Min: 41.53 fg/cell Max: 165.89 fg/cell	Orr and Jones (1998)	Data digitized from Figure 5; Mean calculated using quota data presented for each treatment
	Microcystis aeruginosa	MASH01-A19	Lab	Mean: 93.92 fg/cell Min: 46.58 fg/cell Max: 138.47 fg/cell	Orr and Jones (1998)	Data digitized from Figure 5; Mean calculated using quota data presented for each treatment; Study provides highest reported mean and maximum cell quota value for *Microcystis aeruginosa* mass per cell, lab
	Microcystis aeruginosa	PCC 7806	Lab	Min: 34.5 fg/cell Max: 81.4 fg/cell	Wiedner et al. (2003)	Mean quota value not reported, however data could be digitized from Figure 1B to calculate a mean

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
	Microcystis aeruginosa	Lake Rotura, New Zealand	Lab	Mean: 0.064 pg/cell Min: 0.017 pg/cell Max: 0.134 pg/cell	Wood et al. (2012)	Data digitized from Figure 1B; Mean calculated using cell quota data from all time points; Study provides minimum cell quota value for *Microcystis aeruginosa* mass per cell, lab
	Microcystis aeruginosa	Ontario, Canada	Lab	Min: 40.3 fg/cell Max: 62.4 fg/cell	Pineda-Mendoza et al. (2014)	The range of quota data presented was assumed to be the minimum and maximum values
	Microcystis aeruginosa	New Mexico, United States	Lab	Min: 34.5 fg/cell Max: 136.3 fg/cell	Pineda-Mendoza et al. (2014)	The range of quota data presented was assumed to be the minimum and maximum values
	Microcystis aeruginosa	Umia River, Galicia, Spain	Lab	Mean: 11 µg/g biomass	Alvarez et al. (2016)	Study provides lowest mean and minimum cell quota value for *Microcystis* spp. mass per cell, field, and field and lab combined
	Microcystis aeruginosa	Dayet Afourgah lake, Morocco	Lab	Max: 688.4 µg/g biomass	Douma et al. (2017)	Maximum reported as total microcystins content
	Microcystis aeruginosa	Aguelmam Azigza lake, Morocco	Lab	Max: 699 µg/g biomass	Douma et al. (2017)	Maximum reported as total microcystins content
	Microcystis aeruginosa	Aguelmam Azigza lake, Morocco	Lab	Max: 859.6 µg/g biomass	Douma et al. (2017)	Maximum reported as total microcystins content
	Microcystis aeruginosa	Lake Erie, United States	Lab	Mean: 2.44 µg/mg biomass	Horst et al. (2014)	Data digitized from Figure 5; Study provides highest mean and maximum cell quota value for *Microcystis aeruginosa* mass per biomass, lab
	Microcystis aeruginosa, M. flos-aquae, M. novacekii	Cogotas, Spain	Field	Min: 1.2 pg/cell Max: 4.3 pg/cell	Cires et al. (2013)	Data digitized from Figure 1; Study provides maximum cell quota value for *Microcystis* spp. Mass per

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
						cell, field, and field and lab combined
	Microcystis aeruginosa, M. flos-aquae, M. novacekii	Valmayor, Spain	Field	Min: 3.4 pg/cell Max: 4.1 pg/cell	Cires et al. (2013)	Data digitized from Figure 1; Study provides maximum cell quota value for *Microcystis* spp. mass per cell, field, and field and lab combined
	Microcystis aeruginosa, M. flos-aquae, M. viridis, M. wesenbergii	Lake Taihu, China	Field	Mean: 0.027 pg/cell Min: 0.001 pg/cell Max: 0.087 pg/cell	Tao et al. (2012)	Data digitized from Figure 2c; Mean calculated using all cell quota data for all time points
	Fisherella	NQAIF311 from Queensland, Australia	Lab	Max: 43 µg/g biomass	Cires et al. (2014)	Data digitized from Figure 1
	Geitlerinema	Florida, United States	Field	Min: 0.02 µg/g biomass Max: 0.10 µg/g biomass	Gantar et al. (2009)	
	Geitlerinema	Florida, United States	Lab	Mean: 0.40 µg/g biomass Min: 0.15 µg/g biomass Max: 0.30 µg/g biomass	Gantar et al. (2009)	
	Leptolyngbya	Florida, United States	Field	Min: 0 µg/g biomass Max: 0.08 µg/g biomass	Gantar et al. (2009)	
	Leptolyngbya	FLK BBD1; Florida, United States	Lab	Mean: 0.10 µg/g biomass Min: 0.06 µg/g biomass Max: 0.20 µg/g biomass	Gantar et al. (2009)	
	Phormidium	Florida, United States	Field	Mean: 0.026 µg/g biomass	Gantar et al. (2009)	

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
	Planktothrix spp.	Occhito, Italy	Field	Median: 3.82 µg/mm^3 biovolume Min: 1.27 µg/mm^3 biovolume Max: 6.28 µg/mm^3 biovolume	Salmaso et al. (2014)	Data on minimum and maximum digitized from Figure 4a
	Planktothrix spp.	Pusiano, Italy	Field	Median: 0.59 µg/mm^3 biovolume Min: 0.37 µg/mm^3 biovolume Max: 0.87 µg/mm^3 biovolume	Salmaso et al. (2014)	Data on minimum and maximum digitized from Figure 4a
	Planktothrix spp.	Ledro, Italy	Field	Mean: 0.45 µg/mm^3 biovolume Min: 0.12 µg/mm^3 biovolume Max: 0.84 µg/mm^3 biovolume	Salmaso et al. (2014)	Data on minimum and maximum digitized from Figure 4a
	Planktothrix spp.	Garda, Italy	Field	Mean: 0.31 µg/mm^3 biovolume Min: 0 µg/mm^3 biovolume Max: 0.32 µg/mm^3 biovolume	Salmaso et al. (2014)	Data on minimum and maximum digitized from Figure 4a
	Planktothrix agardhii	Bassenwaithe Lake, England	Field	Mean: 91.2 fg/cell	Akcaalan et al. (2006)	
	Planktothrix agardhii	NIES 595	Lab	Mean: 75.6 fg/cell	Akcaalan et al. (2006)	
	Planktothrix rubescencs	Iznik Lake, Turkey	Field	Mean: 235.6 fg/cell	Akcaalan et al. (2006)	Study provides mean and maximum cell quota value for *Planktothrix rubescencs* mass per cell, field
	Planktothrix rubescencs	France	Field	Min: 0.13 pg/cell Max: 0.16 pg/cell	Briand et al. (2008)	Study provides maximum cell quota value for

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
						Planktothrix rubescencs mass per cell, field, and lab and the minimum cell quota value for mass per cell, field
	Planktothrix rubescencs	SL 03; Turkey	Lab	Mean: 103.9 fg/cell	Akcaalan et al. (2006)	Study provides lowest mean and minimum cell quota value for *Planktothrix rubescencs* mass per cell, lab, and field and lab combined
	Planktothrix rubescencs	Sapanca Lake, Turkey	Lab	Mean: 108.2 fg/cell	Akcaalan et al. (2006)	
	Pseudanabaena	Florida, United States	Field	Min: 0.02 µg/g biomass Max: 0.04 µg/g biomass	Gantar et al. (2009)	
	Spirulina	Florida, United States	Field	Mean: 0.12 µg/g biomass	Gantar et al. (2009)	
	Synechococcus	Florida, United States	Field	Min: 0.08 µg/g biomass Max: 0.27 µg/g biomass	Gantar et al. (2009)	
	Multiple genera including *Microcystis aeruginosa, Anabaenopsis*	Kiwah Island pond, South Carolina	Field		Greenfield et al. (2014)	Data available but were not digitized
	Multiple genera including *Microcystis* spp., *Anabaena* spp., and *Planktolyngbya* spp.	Lake Victoria, Tanzania	Field		Mbonde and Kurmayer (2015)	Data available but were not digitized
	Microcystis, Aphanomenizon, and others	Quebec lakes, Canada	Field		Monchamp et al. (2014)	Data available but were not digitized

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
	Multiple genera including *Microcystis* and *Anabaena*	Anzali wetland, Iran	Field		Rezaitabar et al. (2017)	Data available but were not digitized
	Multiple genera including *Microcystis* and *Anabaena*	Anzali wetland, Iran	Field		Rezaitabar et al. (2017)	Data available but were not digitized
	Multiple genera including *Microcystis, Dolichospermum,* others	Lake Chaohu, China	Field		Shang et al. (2015)	Data available but were not digitized
Cylindrospermopsin	*Aphanizomenon ovalisporum*	Florida, United States	Lab	Min: 7.39 µg/mg biomass Max: 9.33 µg/mg biomass	Yilmaz et al. (2008)	
	Cylindrospermopsis raciborskii[d]	Gazam Dam Lake, Saudi Arabia	Field	Min: 0.6 pg/cell Max: 14.6 pg/cell	Mohamed and Al-Shehri (2013)	Study provides maximum value for *Cylindrospermopsis raciborskii* mass per cell, field, and field and lab combined
	Cylindrospermopsis raciborskii	Queensland, Australia	Field	Mean: 23.12 fg/cell Median: 20.5 fg/cell Min: 5.9 fg/cell Max: 55.8 fg/cell	Orr et al. (2010)	Study provides minimum value for *Cylindrospermopsis raciborskii* mass per cell, field, and field and lab combined
	Cylindrospermopsis raciborskii	Queensland, Australia	Field	Median: 20.3 fg/cell Min: 10 fg/cell Max: 49.4 fg/cell	Orr et al. (2010)	
	Cylindrospermopsis raciborskii	CYP 030A; Australia	Lab	Min: 3.2 ng/10^6 cell Max: 5.7 ng/10^6 cell	Carneiro et al. (2013)	
	Cylindrospermopsis raciborskii	CYP 011K; Australia	Lab	Min: 12.1 ng/10^6 cell	Carneiro et al. (2013)	

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
				Max: 24.7 ng/10^6 cell		
	Cylindrospermopsis raciborskii	Queensland, Australia	Lab	Min: 13.4 fg/cell Max: 14.9 fg/cell	Davis et al. (2014)	
	Cylindrospermopsis raciborskii	New South Wales, Australia	Lab	Mean: 31 fg/cell Min: 12 fg/cell Max: 52 fg/cell	Hawkins et al. (2001)	
	Cylindrospermopsis raciborskii	Queensland, Australia	Lab	Min: 19 fg/cell Max: 26 fg/cell	Pierangelini et al. (2015)	
	Cylindrospermopsis raciborskii	CS-506; Queensland, Australia	Lab	Mean: 0.0028 pg/cell	Willis et al. (2015)	Study provides lowest mean value for *Cylindrospermopsis raciborskii* mass per cell, lab, and field and lab combined and minimum cell quota value for mass per cell, lab
	Cylindrospermopsis raciborskii	CS-506; Queensland, Australia	Lab	Mean: 0.018 pg/cell	Willis et al. (2015)	
	Cylindrospermopsis raciborskii	Lake Wivenhoe, Australia	Lab	Mean: 165.75 fg/cell	Willis et al. (2016)	Calculated mean based on data in Table 1; Study provides highest mean value for *Cylindrospermopsis raciborskii* mass per cell, lab, and field and lab combined and maximum cell quota value for mass per cell, lab
	Cylindrospermopsis raciborskii	CHAB3438, China	Lab	Mean: 43.76 fg/cell Min: 35.89 fg/cell Max: 52 fg/cell	Yang et al. (2016)	Data digitized from Figure 2; Mean calculated using quota data presented for each time point
	Cylindrospermopsis raciborskii	Queensland, Australia	Lab	Min: 416 fg/μm^3 biovolume Max: 447 fg/μm^3 biovolume	Pierangelini et al. (2015)	

Toxin	Genus/Species[a]	Site/Clone	Study Type[b]	Toxin Quota Data[c]	Reference	Notes
	Multiple genera including *Aphanizomenon*, *Anabaena*, *Nostocales*, and *Cylindrospermopsis*	Germany	Field		Rücker et al. (2007)	Data available but were not digitized
	Multiple genera including *Aphanizomenon*	Langer See, Germany	Field		Wiedner et al. (2008)	Data available but were not digitized

Abbreviations: *M*. = *Microcystis*; spp. = multiple species in the genus

[a] Both the genus and species are reported where available. In some studies, the genus was reported but the species was not reported. In other studies, multiple species were analyzed within a specific genus but the specific species were not identified. In both instances, studies were categorized as the genus name (e.g., *Microcystis*) spp. Separately, in some studies multiple genera were considered. In these studies, available toxin quota data were not digitized as they could not be used for comparison purposes. Only information about the studies are presented in this table with a note that data are available but were not digitized.

[b] Studies were conducted in two different settings: the field (i.e., environmental) or a laboratory. In some instances, field samples were subjected to optimized growth conditions in the laboratory. These studies were classified as laboratory; not as field studies.

[c] Toxin cell quota data were not converted and are reported in the measurement units used by the study authors. Significant figures were not normalized among the data points.

[d] The genus *Cylindrospermopsis* has been renamed to *Raphidiopsis*.

Table G-4 provides the first step in summarizing and grouping cell quota data for microcystin and cylindrospermopsin-producing genera. Studies presented in Table G-3 were grouped by genus and species when possible. Studies that looked at more than one species within a specific genus or that did not specify which species were considered within that genus were placed in a single group (e.g., *Microcystis* spp., *Planktothrix* spp.). Within each genus/species group, studies were further grouped based on their study type and the quantification method used in that study. For each study type and quantification method group, data were aggregated on the mean, minimum, and maximum cell quota values presented in each study included in that group. In Table G-4, the range of the means, arithmetic mean (of the means), median of the means, minimum cell quota value, and maximum cell quota value are reported for the studies included in that group. Note that studies were not identified in the literature search for all quantification methods and study types for all genus/species groups. The EPA converted data to a standard set of units, pg per cell, when possible. No other conversions were attempted. Additional information about the approach used to summarize the available cell quota data is provided in the footnotes accompanying the table.

Table G-4. Cell Quota Appendix Summary Data for Microcystin and Cylindrospermopsin-producing Genera

Toxin	Genus, Species	Quantification Method[a]; Study Type[b]	Range of Means[c,d]	Mean[c,d]	Median of Means[c,e]	Minimum; Maximum[c,f]	References
Microcystin	*Microcystis* spp.	Mass per cell; Field and lab	0.015–0.576 pg/cell	0.13 pg/cell	0.017 pg/cell	0 pg/cell; 4.30 pg/cell	Sitoki et al. (2012); Tao et al. (2012); Cires et al. (2013); Sabart et al. (2013); Wang et al. (2013); Wei et al. (2016)
		Mass per cell; Field	0.015–0.576 pg/cell	0.16 pg/cell	0.022 pg/cell	0 pg/cell; 4.30 pg/cell	Sitoki et al. (2012); Tao et al. (2012); Cires et al. (2013); Sabart et al. (2013); Wang et al. (2013)
		Mass per cell; Lab	0.020 pg/cell	0.02 pg/cell	N/A	0.017 pg/cell; 0.028 pg/cell	Wei et al. (2016)
		Mass per biomass; Field	640.59 µg/g biomass	640.59 µg/g biomass	N/A	13.21 µg/g; 1389.13 µg/g biomass	Alvarez et al. (2016); Wei et al. (2016)
	Microcystis aeruginosa	Mass per cell; Field and lab	0.02–0.14 pg/cell	0.09 pg/cell	0.09 pg/cell	0.01 pg/cell; 0.35 pg/cell	Orr and Jones (1998); Jähnichen et al. (2001); Wiedner et al. (2003); Jähnichen et al. (2007); Fahnenstiel et al. (2008); Vasconcelos et al. (2011); Wood et al. (2012); Pineda-Mendoza et al. (2014); Chia et al. (2016)
		Mass per cell; Field	0.12–0.14 pg/cell	0.13 pg/cell	0.13 pg/cell	0.01 pg/cell; 0.35 pg/cell	Fahnenstiel et al. (2008); Vasconcelos et al. (2011)
		Mass per cell; Lab	0.02–0.09 pg/cell	0.07 pg/cell	0.08 pg/cell	0.02 pg/cell; 0.17 pg/cell	Orr and Jones (1998); Jähnichen et

Toxin	Genus, Species	Quantification Method[a]; Study Type[b]	Range of Means[c,d]	Mean[c,d]	Median of Means[c,e]	Minimum; Maximum[c,f]	References
							al. (2001); Wiedner et al. (2003); Jähnichen et al. (2007); Wood et al. (2012); Pineda-Mendoza et al. (2014); Chia et al. (2016)
		Mass per biomass; Field	3,340 µg/g biomass	3,340 µg/g biomass	N/A	0.14 µg/g biomass; 3,340 µg/g biomass	Mbukwa and Mamba (2012); Horst et al. (2014)
		Mass per biomass; Lab	11–2,440 µg/g biomass	1225.5 µg/g biomass	1225.5 µg/g biomass	11 µg/g; 2,440 µg/g biomass	Horst et al. (2014); Alvarez et al. (2016); Douma et al. (2017)
	Fisherella	Mass per biomass; Lab	N/A	N/A	N/A	43 µg/g biomass	Gantar et al. (2009)
	Geitlerinema	Mass per biomass; Field	N/A	N/A	N/A	0.02 µg/g; 0.10 µg/g biomass	Gantar et al. (2009)
		Mass per biomass; Lab	0.40 µg/g biomass	0.40 µg/g biomass	N/A	0.15 µg/g; 0.40 µg/g biomass	Gantar et al. (2009)
	Leptolyngbya	Mass per biomass; Field	N/A	N/A	N/A	0 µg/g; 0.08 µg/g biomass	Gantar et al. (2009)
		Mass per biomass; Lab	0.10 µg/g biomass	0.10 µg/g biomass	N/A	0.06 µg/g; 0.20 µg/g biomass	Gantar et al. (2009)
	Phormidium	Mass per biomass; Lab	0.026 µg/g biomass	0.026 µg/g biomass	N/A	0.026 µg/g biomass	Gantar et al. (2009)
	Planktothrix spp.	Mass per biovolume; Field	N/A	N/A	N/A	0 µg/mm^3; 6.28 µg/mm^3 biomass	Salmaso et al. (2014)

Toxin	Genus, Species	Quantification Method[a]; Study Type[b]	Range of Means[c,d]	Mean[c,d]	Median of Means[c,e]	Minimum; Maximum[c,f]	References
	Planktothrix agardhii	Mass per cell; Field and lab	0.076–0.091 pg/cell	0.083 pg/cell	0.083 pg/cell	0.076 pg/cell; 0.091 pg/cell	Akcaalan et al. (2006)
		Mass per cell; Field	0.091 pg/cell	0.091 pg/cell	N/A	0.091 pg/cell	Akcaalan et al. (2006)
		Mass per cell; Lab	0.076 pg/cell	0.076 pg/cell	N/A	0.076 pg/cell	Akcaalan et al. (2006)
	Planktothrix rubescencs	Mass per cell; Field and lab	0.104–0.236 pg/cell	0.149 pg/cell	.108 pg/cell	0.104 pg/cell; 0.16 pg/cell	Akcaalan et al. (2006); Briand et al. (2008)
		Mass per cell; Field	0.236 pg/cell	0.236 pg/cell	N/A	0.13 pg/cell; 0.236 pg/cell	Akcaalan et al. (2006); Briand et al. (2008)
		Mass per cell; Lab	0.104–0.108 pg/cell	0.106 pg/cell	0.106 pg/cell	0.104 pg/cell; 0.108 pg/cell	Akcaalan et al. (2006)
	Pseudanabaena	Mass per biomass; Field	N/A	N/A	N/A	0.02 µg/g; 0.04 µg/g biomass	Gantar et al. (2009)
	Spirulina	Mass per biomass; Field	0.12 µg/g biomass	0.12 µg/g biomass	N/A	0.12 µg/g biomass	Gantar et al. (2009)
	Synechococcus	Mass per biomass; Field	N/A	N/A	N/A	0.08 µg/g; 0.27 µg/g biomass	Gantar et al. (2009)
Cylindrospermopsin	*Aphanizomenon ovalisporum*	Mass per biomass; Field and lab	N/A	N/A	N/A	7.39 µg/g ; 9.33 µg/mg biomass	Yilmaz et al. (2008)
	Cylindrospermopsis raciborskii[g]	Mass per cell; Field and lab	0.0028–0.17 pg/cell	0.05 pg/cell	0.03 pg/cell	0.006 pg/cell; 14.6 pg/cell	Orr et al. (2010); Mohamed and Al-Shehri (2013); Pierangelini et al. (2015); Willis et al. (2015); Yang et al. (2016a)

Toxin	Genus, Species	Quantification Method[a]; Study Type[b]	Range of Means[c,d]	Mean[c,d]	Median of Means[c,e]	Minimum; Maximum[c,f]	References
		Mass per cell; Field	0.023 pg/cell	0.023 pg/cell	N/A	0.006 pg/cell; 14.6 pg/cell	Orr et al. (2010); Mohamed and Al-Shehri (2013)
		Mass per cell; Lab	0.0028–0.17 pg/cell	0.057 pg/cell	0.031	0.0028 pg/cell; 0.17 pg/cell	Hawkins et al. (2001); Carneiro et al. (2013); Davis et al. (2014); Pierangelini et al. (2015); Willis et al. (2015); Willis et al. (2016); Yang et al. (2016)
		Mass per biovolume; Lab	N/A	N/A	N/A	416 fg/μm^3; 447 fg/μm^3	Pierangelini et al. (2015)

Acronyms and Abbreviations: fg = femtogram; pg = picogram; µg = microgram; N/A = not applicable.

[a] Various methods were used to quantify toxin quotas and quota values were presented in different forms, including toxin mass per cyanobacterial cell and toxin mass per cyanobacterial biomass.

[b] Studies were conducted in two different settings: the field (i.e., environmental) or a laboratory. In some instances, field samples were subjected to optimized growth conditions in the laboratory. These studies were classified as laboratory; not field.

[c] Study authors reported data using multiple measurement units. When possible, the EPA converted data to the standard units of pg per cell. The EPA did not identify appropriate conversion factors that would allow genus-specific conversion of quotas described in mass per biomass to mass per cell.

[d] Shows single reported mean if only one study was available or average of reported means.

[e] Median of means not calculated if only one mean value was available or if only minimum and/or maximum cell quota values were available.

[f] If reported toxin quota means from one study were the lowest or highest toxin quotas reported within a genus, then these values were listed as the minimum or maximum values, respectively, to better reflect the range of toxin quota values.

[g] The genus *Cylindrospermopsis* has recently been renamed to *Raphidiopsis*.

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APPENDIX H. TABLES OF STATE-ISSUED GUIDELINES SPECIFIC TO ANIMAL CYANOTOXIN POISONING

H.1 California

Table H-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 H-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-day	0.085	0.085
Uncertainty factor (unitless)	3	3
Acute RfD[a] mg/kg/day	0.037	0.04
Acute action level µg/L	100	200
Subchronic RfD mg/kg/day	0.00064	0.0033
Subchronic action level µg/L	2	10

Reference:

Butler N, Carlisle J, Kaley KB, and 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: 11/27/2018.

H.2 Indiana

Indiana has adopted guidance for cyanotoxins for dog exposures:

"A warning to dog owners using the Fort Harrison State Park Dog Park Lake will occur whenever any cyanotoxins are detected, and dogs will be prohibited from swimming at the values of 0.8 µg/L microcystin, any anatoxin-a detection, and 1.0 µg/L of cylindrospermopsin."

Reference:

Indiana Department of Environmental Management (2018). Blue-Green Algae: Indiana Reservoir and Lake Update.http://www.in.gov/idem/algae/. Last Accessed: 02/27/2018.

H.3 Oregon

Table H-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 (2018). Oregon Harmful Algae Bloom Surveillance (HABS) Program Public Health Advisory Guidelines: Harmful Algae Blooms in Freshwater Bodies. https://www.oregon.gov/oha/ph/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Documents/HABPublicHealthAdvisoryGuidelines.pdf.

H.4 Grayson County, Texas

Table H-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 microcystin concentration 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.
**LD_{50} for microcystin-mouse used in calculations = 45 µg/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	2,200
80-pound dog	2,109	17,601

*This is not including additional dose amounts that could be ingested from a dog self-grooming algae scum off its fur.
**LD_{50} for cylindrospermopsin-mouse used in calculations = 4400 µg/kg
***20 ppb cylindrospermopsin is algal toxin threshold for BGA Warning (condition red)

Reference:

Lillis J, Ortez A, and Teel JH (2012). *Blue-Green Algae Response Strategy*. Sherman, Texas. http://www.co.grayson.tx.us/upload/page/0206/docs/Blue-Green_Algae_Response_Strategy.pdf. Last Accessed: 12/5/2018.