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Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins

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ABBREVIATIONS AND ACRONYMS

Γgt	Γ-Glutamyltransferase
A	Alanine
Adda	3-Amino-9-Methoxy-2,-6,-8-,Trimethyl-10-Phenyldeca-4,-6-Dienoic Acid
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
AWWARF	American Water Works Association Research Foundation
BMD	Benchmark Dose
BMDL	Benchmark Dose Level
BW	Body Weight
Cl ₂	Chlorine Dioxide
CAS	Chemical Abstracts Service
CASA	Computer-Assisted Sperm Analysis
CCL	Contaminant Candidate List
CWA	Clean Water Act
DBP	Disinfection By-Products
DL	Detection Limit
EBCT	Empty Bed Contact Time
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	U.S. Environmental Protection Agency
FSH	Follicle Stimulating Hormone
g	Gram
GC/MS	Gas Chromatograph/Mass Spectrometry
GAC	Granular Activated Carbon
GLERL	Great Lakes Environmental Research Laboratory
H ₂ O ₂	Hydrogen Peroxide
HA	Health Advisory
HAB	Harmful Algal Bloom
HESD	Health Effects Support Document
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
Kg	Kilogram
L	Leucine
LC/MS	Liquid Chromatography/Mass Spectrometry
LDH	Lactate Dehydrogenase
LD ₅₀	Lethal Dose to 50% of Organisms
LH	Luteinizing Hormone
LOAEL	Lowest-Observed-Adverse-Effect Level
MC-LA	Microcystin-LA
MC-LR	Microcystin-LR
MC-RR	Microcystin-RR
MC-YR	Microcystin-YR
MC-YM	Microcystin-YM

Mdha	Methyldehydroalanine
MERHAB-LGL	Monitoring and Event Response to Harmful Algal Blooms in the Lower Great Lakes
µg	Microgram
µm	Micromole
LOQ	Level of Quantification
Mdls	Method Detection Limit
mg	Milligram
ml	Milliliter
MMPB	2-methyl-3-methoxy-4-phenylbutyric acid
MOA	Mode of Action
MF	Microfiltration
MWCO	Molecular Weight Cut-Off
NDEA	N-Nitrosodiethylamine
NF	Nanofiltration
NLA	National Lakes Assessment
NOAA	National Oceanic and Atmospheric Administration
NOAEL	No-Observed-Adverse-Effect Level
NOD	Nodularin
NOM	Natural Organic Material
OATp	Organic Acid Transporter Polypeptides
PAC	Powdered Activated Carbon
PAS	Periodic Acid-Schiff
PBS	Phosphate-Buffered Saline
PDA	Photodiode Array Detector
P-GST	glutathione <i>S</i> -transferase placental form-positive
POD	Point of Departure
POU	Point-of-Use
PP2	Protein Phosphatase 2A
PP1	Protein Phosphatase 1
PPIA	Protein Phosphatase Inhibition Assays
RfD	Reference Dose
RO	Reverse Osmosis
ROS	Reactive Oxygen Species
SDWA	Safe Drinking Water Act
SPE	Solid-Phase Extraction
TEF	Toxicity Equivalency Factors
TOC	Total Organic Carbon
TOXLINE	Toxicology Literature Online
TUNEL	Terminal Deoxynucleotidyl Transferase-Mediated dUTP-Biotin Nick End-Labeling Assay
UF	Uncertainty Factor
UF	Ultrafiltration
USGS	United States Geological Survey
UV	Ultraviolet
WHO	World Health Organization

EXECUTIVE SUMMARY

Microcystins are toxins produced by a number of cyanobacteria species, including members of *Microcystis*, *Anabaena*, *Nodularia*, *Nostoc*, *Oscillatoria*, *Fischerella*, *Planktothrix*, and *Gloeotrichia*. Approximately 100 microcystin congeners exist, which vary based on amino acid composition. Microcystin-LR is one of the most potent congeners and the majority of toxicological data on the effects of microcystins are available for this congener.

Many environmental factors such as the ratio of nitrogen to phosphorus, temperature, organic matter availability, light attenuation and pH play an important role in the development of microcystin blooms, both in fresh and marine water systems and could encourage toxin production. Microcystins are water soluble and tend to remain contained within the cyanobacterial cell (intracellular), until the cell breaks and they are released into the water (extracellular).

This Health Advisory (HA) for microcystins is focused on drinking water as the primary source of exposure. Exposure to cyanobacteria and their toxins may also occur by ingestion of toxin-contaminated food, including consumption of fish, and by inhalation and dermal contact during bathing or showering and during recreational activities in waterbodies with the toxins. While these types of exposures cannot be quantified at this time, they are assumed to contribute less to the total cyanotoxin exposures than ingestion of drinking water. Due to the seasonality of cyanobacterial blooms, exposures are not expected to be chronic.

Limited data in humans and animals demonstrate the absorption of microcystins from the intestinal tract and distribution to the liver, brain, and other tissues. Elimination from the body requires facilitated transport using receptors belonging to the Organic Acid Transporter polypeptide (OATp) family. Data for humans and other mammals suggest that the liver is a primary site for binding these proteins (i.e., increased liver weight in laboratory animals and increased serum enzymes in laboratory animals and humans). Once inside the cell, these toxins covalently bind to cytosolic proteins (PP1 and PP2) resulting in their retention in the liver. Limited data are available on the metabolism of microcystins, but most of the studies indicate that microcystins can be conjugated with glutathione and cysteine to increase their solubility and facilitate excretion.

The main source of human health effects data for microcystins is from acute recreational exposure to cyanobacterial blooms. Symptoms include headache, sore throat, vomiting and nausea, stomach pain, dry cough, diarrhea, blistering around the mouth, and pneumonia. However, human data on the oral toxicity of microcystins are limited and confounded by: potential co-exposure to other contaminants; a lack of quantitative information; and other confounding factors. Reports of human intravenous exposure to dialysate prepared with microcystin-contaminated water indicated acute liver failure and death in a large number of the exposed patients.

Studies in laboratory animals demonstrate liver, kidney, and reproductive effects following short-term and subchronic oral exposures to microcystin-LR. Studies evaluating the chronic toxicity of microcystins have not shown clinical signs of toxicity and are limited by study design and by the lack of quantitative data.

The U.S. Environmental Protection Agency (EPA) identified a study by Heinze (1999) conducted on rats as the critical study used in the derivation of the reference dose (RfD) for microcystins. The critical effects identified in the study are increased liver weight, slight to moderate liver lesions with hemorrhages, and increased enzyme levels as a result of exposure to microcystin-LR. The lowest-observed-adverse-effect level (LOAEL) was determined to be 50 µg/kg/day, based on these effects. The drinking water route of exposure matches potential drinking water exposure scenarios in humans. The total uncertainty factor (UF) applied to the LOAEL was 1000. This was based on a UF of 10 for intraspecies variability, a UF of 10 for interspecies variability, a UF of 3 (10^{1/2}) for extrapolation from a LOAEL to no-observed-adverse-effect level (NOAEL), and a UF of 3 (10^{1/2}) to account for deficiencies in the database. EPA is using microcystin-LR as a surrogate for other microcystin congeners. Therefore, the HA based on this critical study applies to total microcystins.

EPA is issuing a Ten-day HA for microcystins based on the Heinze (1999) short-term, 28-day study. Studies of a duration of 7 to 30 days are typically used to derive Ten-day HAs. The HA is consistent with this duration and appropriately matches human exposure scenarios for microcystins in drinking water. Cyanobacterial blooms are usually seasonal, typically occurring from May through October. Microcystins typically have a half-life of 4 days to 14 days in surface waters, (depending on the degree of sunlight, natural organic matter, and the presence of bacteria) and can be diluted via transport. In addition, concentrations in finished drinking water can be reduced by drinking water treatment and management measures.

The Ten-day HA value for bottle-fed infants and young children of pre-school age is 0.3 µg/L and for school-age children through adults is 1.6 µg/L for microcystins. The two advisory values use the same toxicity data (RfD) and represent differences in drinking water intake and body weight for different life stages. The first advisory value is based on the summation of the time-weighted drinking water intake/body weight ratios for birth to <12 months of age. The second advisory value is based on the mean body weight and 90th percentile drinking water consumption rates for adults age 21 and over (U.S. EPA's Exposure Factors Handbook (2011a)), which is similar to that of school-aged children. Populations such as pregnant women and nursing mothers, the elderly, and immune-compromised individuals or those receiving dialysis treatment may be more susceptible than the general population to the health effects of microcystins. As a precautionary measure, individuals that fall into these susceptible groups may want to consider following the recommendations for children pre-school age and younger. This HA is not a regulation, it is not legally enforceable, and it does not confer legal rights or impose legal obligations on any party.

Applying the U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment, there is *inadequate information to assess carcinogenic potential* of microcystins. The few available epidemiological studies are limited by their study design, poor measures of exposure, potential co-exposure to other contaminants, and the lack of control for confounding factors. No long term animal studies were available to evaluate dose-response for the tumorigenicity of microcystins following lifetime exposures. Other studies evaluating the tumor promotion potential of microcystin found an increase in the number and/or size of GST-P positive foci observed. In two promotion studies, microcystin-LR alone showed no initiating activity.

1.0 INTRODUCTION AND BACKGROUND

EPA developed the non-regulatory Health Advisory (HA) Program in 1978 to provide information for public health officials or other interested groups on pollutants associated with short-term contamination incidents or spills for contaminants that can affect drinking water quality, but are not regulated under the Safe Drinking Water Act (SDWA). At present, EPA lists HAs for 213 contaminants (<http://water.epa.gov/drink/standards/hascience.cfm>).

HAs identify the concentration of a contaminant in drinking water at which adverse health effects are not anticipated to occur over specific exposure durations (e.g., one-day, ten-days, and a lifetime). HAs serve as informal technical guidance to assist Federal, State and local officials, and managers of public or community water systems in protecting public health when emergency spills or contamination situations occur. An HA provides information on the environmental properties, health effects, analytical methodology, and treatment technologies for removal of drinking water contaminants.

The *Health Effects Support Document for Microcystins* (U.S.EPA, 2015a) is the peer-reviewed, effects assessment that supports this HA. This document is available at <http://www2.epa.gov/nutrient-policy-data/health-and-ecological-effects>. The HAs are not legally enforceable Federal standards and are subject to change as new information becomes available. The structure of this Health Advisory is consistent with EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (U.S.EPA, 2014).

EPA is releasing the *Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water* (U.S. EPA, 2015b) as a companion to the HAs for microcystins and cylindrospermopsin. The document is intended 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. It is designed to provide information and a framework that PWSs and others as appropriate may consider to inform their decisions on managing the risks from cyanotoxins in drinking water.

1.1 Current Criteria, Guidance and Standards

Currently there are no U.S. federal water quality criteria, or regulations for cyanobacteria or cyanotoxins in drinking water under the SDWA or in ambient waters under the Clean Water Act (CWA). The Safe Drinking Water Act (SDWA), as amended in 1996, requires the EPA to publish a list of unregulated contaminants every five years that are not subject to any proposed or promulgated national primary drinking water regulations, which are known or anticipated to occur in public water systems, and which may require regulation. This list is known as the Contaminant Candidate List (CCL). The EPA's Office of Water included cyanobacteria and cyanotoxins on the first and second CCL (CCL 1, 1998; CCL 2, 2005). EPA included cyanotoxins, including anatoxin-a, cylindrospermopsin, and microcystin-LR, on CCL 3 (2009) and the draft CCL 4 (April 2015 for consideration).

SDWA requires the Agency to make regulatory determinations on at least five CCL contaminants every five years. When making a positive regulatory determination, EPA determines whether a contaminant meets three criteria:

- The contaminant may have an adverse effect on the health of persons,
- The contaminant is known to occur or there is substantial likelihood the contaminant will occur in public water systems with a frequency and at levels of concern, and
- In the sole judgment of the Administrator, regulating the contaminant presents a meaningful opportunity for health risk reductions.

To make these determinations, the Agency uses data to analyze occurrence (prevalence and magnitude) and health effects. EPA continues gathering this information to inform future regulatory determinations for cyanotoxins under the SDWA. The SDWA also provides the authority for EPA to publish non-regulatory HAs or take other appropriate actions for contaminants not subject to any national primary drinking water regulation. EPA is providing this HA and the HA for cylindrospermopsin to assist State and local officials in evaluating risks from these contaminants in drinking water.

Internationally, eighteen countries and three U.S. states have developed drinking water guidelines for microcystins, as shown in Table 1.1 and Table 1.2, respectively, based on lifetime exposures.

Table 1-1. International Guideline Values for Microcystins

Country	Guideline Value	Source
Brazil, China, Czech Republic, Denmark, Finland, France, Germany, Italy, Japan, Korea, Netherlands, Norway, New Zealand, Poland, South Africa, and Spain	1.0 µg/L microcystin-LR	Based on the World Health Organization (WHO) Provisional Guideline Value of 1µg/L for drinking water (WHO, 1999; 2003)
Australia	1.3 µg/L microcystin-LR (toxicity equivalents)	Australian Drinking Water Guidelines 6 (NHMRC, NRMCC, 2011)
Canada	1.5 µg/L microcystin-LR	Guidelines for Canadian Drinking Water Quality: Supporting Documentation Cyanobacterial Toxins- Microcystin-LR (Health Canada, 2002)

Table 1-2. State Guideline Values for Microcystins

State	Guideline Value	Source
Minnesota	0.04 µg/L Microcystin-LR	Minnesota Department of Health (MDH, 2012)
Ohio	1 µg/L Microcystin	Public Water System Harmful Algal Bloom Response Strategy (Ohio EPA, 2014)
Oregon	1 µg/L Microcystin-LR	Public Health Advisory Guidelines, Harmful Algae Blooms in Freshwater Bodies. (OHA, 2015)

For drinking water, the provisional WHO Guideline value for microcystin-LR of 1 µg/L (or the underlying Tolerable Daily Intake (TDI) of 0.04 µg/kg) has been widely used as the basis for national standards or guideline values (WHO, 1999, 2003). Following the release of the WHO provisional guideline, drinking-water standards or national guideline values were adopted in 16 countries. Australia and Canada have used the TDI, but have adapted other factors in the calculation to reflect their national circumstances (e.g. body weight or amounts of water consumed), thus reaching somewhat higher guidance values or standards (Chorus, 2012). A few countries have issued guideline values specifically for microcystin-LR while others use microcystin-LR as a surrogate for all microcystin congeners (i.e. toxicity equivalents). Values are similar across all countries, ranging between 1.0 and 1.5 µg/L based on lifetime exposures.

2.0 PROBLEM FORMULATION

The development of the HA begins with problem formulation, which provides a strategic framework by focusing on the most relevant cyanotoxin properties and endpoints identified in the *Health Effects Support Document for Microcystins* (U.S. EPA, 2015a).

2.1 Cyanobacteria and Production of Microcystins

Cyanobacteria, formerly known as blue-green algae (Cyanophyceae), are a group of bacteria with chlorophyll-a capable of photosynthesis (light and dark phases) (Castenholz and Waterbury, 1989). Most cyanobacteria are aerobic photoautotrophs, requiring only water, carbon dioxide, inorganic nutrients and light for survival, while others have heterotrophic properties and can survive long periods in complete darkness (Fay, 1965). Some species are capable of nitrogen fixation (diazotrophs) (Duy et al., 2000), producing inorganic nitrogen compounds for the synthesis of nucleic acids and proteins. Cyanobacteria can form symbiotic associations with animals and plants, such as fungi, bryophytes, pteridophytes, gymnosperms and angiosperms (Rai, 1990), supporting their growth and reproduction (Sarma, 2013; Hudnell, 2008; Hudnell, 2010).

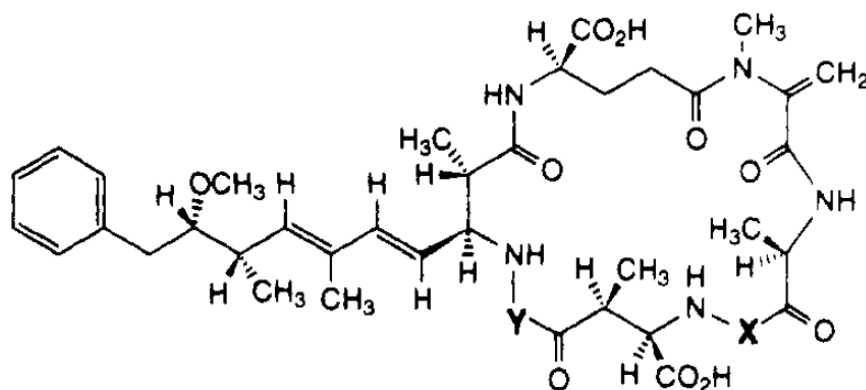
Under the right conditions of pH, nutrient availability, light, and temperature, cyanobacteria can reproduce quickly forming a bloom. Although studies of the impact of environmental factors on cyanotoxin production are ongoing, nutrient (N, P and trace metals) supply rates, light, temperature, oxidative stressors, interactions with other biota (viruses, bacteria and animal grazers), and most likely, the combined effects of these factors are all involved (Paerl and Otten 2013a; 2013b). Fulvic and humic acids reportedly encourage cyanobacteria growth (Kosakowska et al., 2007).

Microcystins are produced by several cyanobacterial species, including *Anabaena*, *Fischerella*, *Gloeotrichia*, *Nodularia*, *Nostoc*, *Oscillatoria*, members of *Microcystis*, and *Planktothrix* (Duy et al., 2000; Codd et al., 2005; Stewart et al., 2006a; Carey et al., 2012).

2.2 Physical and Chemical Properties

The cyclic peptides include around 100 congeners of microcystins. Table 2-1 lists only the most common microcystins congeners. Figure 2-1 provides the structure of microcystin where X and Y represent variable amino acids. Although substitutions mostly occur in positions X and Y, other modifications have been reported for all of the amino acids (Puddick et al., 2015). The amino acids are joined end-to-end and then head to tail to form cyclic compounds that are comparatively large (molecular weights ranging from ~800 to 1,100 g/mole).

Figure 2-1. Structure of Microcystin (Kondo et al., 1992)



Microcystin congeners vary based on their amino acid composition and through methylation or demethylation at selected sites within the cyclic peptide (Duy et al., 2000). The variations in composition and methylation account for the large number of toxin congeners. The microcystins are named based on their variable amino acids, although they have had many other names (Carmichael et al., 1988). For example, microcystin-LR, the most common congener, contains leucine (L) and arginine (R) (Carmichael, 1992). The letters used to identify the variable amino acids are the standard single letter abbreviations for the amino acids found in proteins. The variable amino acids are usually the L-amino acids as found in proteins. In this HA, the term microcystin may be followed by the abbreviations for the variable amino acids. For example, microcystin-LR is for the microcystin with leucine in the X position of Figure 2-1 and arginine in the Y position. Most research has concentrated on microcystin-LR, with lesser amounts of data available for the other amino acid combinations. For the purpose of this HA, microcystin-LR is used as the surrogate for total microcystins.

Structurally, the microcystins are monocyclic heptapeptides that contain seven amino acids: two variable L-amino acids, three common D-amino acids or their derivatives, and two novel D-amino acids. These two D-amino acids are: 3S-amino-9S-methoxy-2,6,8S,-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) and methyldehydroalanine (Mdha). Adda is characteristic of all toxic microcystin structural congeners and is essential for their biological activity (Rao et al., 2002; Funari and Testai, 2008). Mdha plays an important role in the ability of the microcystins to inhibit protein phosphatases. Figure 2-2 illustrates the structures of these two unique amino acid microcystin components.

Microcystins are water soluble. In aquatic environments, the cyclic peptides tend to remain contained within the cyanobacterial cell and are released in substantial amounts only upon cell lysis. The microcystins are most frequently found in cyanobacterial blooms in fresh and brackish waters (WHO, 1999). Table 2-2 provides chemical and physical properties of microcystin-LR.

Table 2-1. Abbreviations for Microcystins (Yuan et al., 1999)

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

Figure 2-2. Structure of the amino acids Adda and Mdha (Harada et al., 1991).

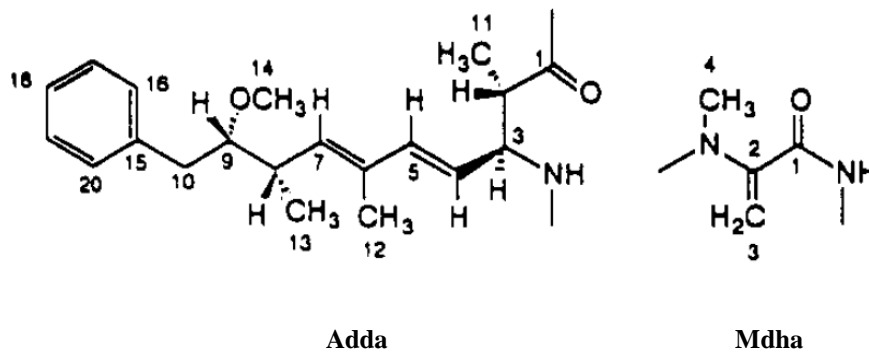


Table 2-2. Chemical and Physical Properties of Microcystin-LR

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

Sources: Chemical Book, 2012; TOXLINE, 2012

2.3 Sources and Occurrence

Cyanotoxin production is strongly influenced by the environmental conditions that promote growth of particular cyanobacterial species and strain. Nutrient concentrations, light intensity, water turbidity, temperature, competing bacteria and phytoplankton, pH, turbulence, and salinity are all factors that affect cyanobacterial growth and change in cyanobacteria population dynamics. Although environmental conditions affect the formation of blooms, the numbers of cyanobacteria and toxin concentrations produced are not always closely related. Cyanotoxin concentrations depend on the dominance and diversity of strains within the bloom along with environmental and ecosystem influences on bloom dynamics (Hitzfeld et al., 2000; Chorus et al., 2000; WHO, 1999). Extracellular microcystins (either dissolved in water or bound to other materials) typically make up less than 30% of the total microcystin concentration in source water (Graham et al., 2010). Most of the toxin is intracellular, and released into the water when the cells rupture or die. Both intracellular and extracellular microcystins may also be present in treated water, depending on the type of treatment processes in place.

2.3.1 Occurrence in Surface Water

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

According to a survey conducted in Florida in 1999 between the months of June and November, the most frequently observed cyanobacteria were *Microcystis* (43.1%), *Cylindrospermopsis* (39.5%), and *Anabaena spp* (28.7%) (Burns, 2008). Of 167 surface water samples taken from 75 waterbodies, 88 samples were positive for cyanotoxins. Microcystin was the most commonly found cyanotoxin in water samples collected, occurring in 87 water samples.

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

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

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

EPA's National Aquatic Resource Surveys (NARS) generate national estimates of pollutant occurrence every 5 years. In 2007, the National Lakes Assessment (NLA) conducted the first-ever national probability-based survey of the nation's lakes, ponds and reservoirs (U.S.EPA, 2009). This baseline study of the condition of the nation's lakes provided estimates of the condition of natural and man-made freshwater lakes, ponds, and reservoirs greater than 10 acres and at least one meter deep. A total of 1,028 lakes were sampled in the NLA during the summer

of 2007. The NLA measured microcystins using Enzyme Linked Immunosorbent Assays (ELISA) with a detection limit of 0.1 µg/L as well as cyanobacterial cell counts and chlorophyll-a concentrations, which were indicators of the presence of cyanobacterial toxins. Samples were collected in open water at mid-lake. Due to the design of the survey, no samples were taken nearshore or in other areas where scums were present.

A total of 48 states were sampled in the NLA, and states with lakes reporting microcystins levels above the WHO's moderate risk¹ threshold in recreational water (>10 µg/L) are shown in Table 2-3. Microcystins were present in 30% of the lakes sampled nationally, with sample concentrations that ranged from the limit of detection (0.1 µg/L) to 225 µg/L. Two states (North Dakota and Nebraska), had 9% of samples above 10 µg/L. Other states including Iowa, Texas, South Dakota, and Utah also had samples that exceeded 10 µg/L. Several samples in North Dakota, Nebraska, and Ohio exceeded the WHO high risk threshold value for recreational waters of 20 µg/L (192 and 225 µg/L, respectively). EPA completed a second survey of lakes in 2012, but data have not yet been published.

Microcystins have been detected in most of the states of the U.S., and over the years many studies have been done to determine their occurrence in surface water. USGS, for example, did a study in the Upper Klamath Lake in Oregon in 2007 and detected total microcystin concentrations between 1 µg/L and 17 µg/L (VanderKooi et al., 2010). USGS also monitored Lake Houston in Texas from 2006 to 2008, and found microcystins in 16% of samples with concentrations less than or equal to 0.2 µg/L (Beussink and Graham, 2011). In 2011, USGS conducted a study on the upstream reservoirs of the Kansas River, a primary source of drinking water for residents in northeastern Kansas, to characterize the transport of cyanobacteria and associated compounds (Graham et al., 2012). Concentrations of total microcystin were low in the majority of the tributaries with the exception of Milford Lake, which had higher total microcystin concentrations, some exceeding the Kansas recreational guidance level of 20 µg/L. Upstream from Milford Lake, a cyanobacterial bloom was observed with a total microcystin concentration of 150,000 µg/L. When sampled a week later, total microcystin concentrations were less than 1 µg/L. The study authors indicated that this may be due to dispersion of microcystins through the water column or to other areas, or by degradation of microcystins via abiotic and biological processes. Samples taken during the same time from outflow waters contained total microcystin concentrations of 6.2 µg/L.

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

¹ The WHO established guideline values for recreational exposure to cyanobacteria using a three-tier approach: low risk (<20,000 cyanobacterial cells/ml corresponding to <10 µg/L of MC-LR); moderate risk (20,000-100,000 cyanobacterial cells/ml corresponding to 10-20 µg/L of MC-LR); and high risk (>100,000 cyanobacterial cells/ml corresponding to >20 µg/L for MC-LR).

Table 2-3. States Surveyed as Part of the 2007 National Lakes Assessment with Water Body Microcystins Concentrations above the WHO Advisory Guideline Level for Recreational Water of 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%	192 µg/L
Nebraska	42	9.1%	225 µg/L
South Dakota	40	4.9%	33 µg/L
Ohio	21	4.5%	78 µg/L*
Iowa	20	4.5%	38 µg/L*
Utah	26	3.6%	15 µg/L*
Texas	51	1.8%	28 µg/L *

* Single Sample

Other surveys and studies have been conducted to determine the occurrence of microcystin in lakes in the United States. A survey conducted during the spring and summer of 1999 and 2000 in more than 50 lakes in New Hampshire found measureable microcystin concentrations in all samples (Haney and Ikawa, 2000). Microcystins were analyzed by ELISA and were found in all of the lakes sampled with a mean concentration of 0.1 µg/L. In 2005 and 2006, a study conducted in New York, including Lake Ontario, found variability in microcystin-LR concentrations within the Lake Ontario ecosystem (Makarewicz et al., 2009). Of the samples taken in Lake Ontario coastal waters, only 0.3% of the samples exceeded the WHO provisional guideline value for drinking water of 1 µg/L. However, 20.4% of the samples taken at upland lakes and ponds within the Lake Ontario watershed, some of them sources of drinking water, exceeded 1 µg/L. During 2008 and 2009, a study was conducted in Kabetogama Lake, Minnesota which detected microcystin concentrations in association with algal blooms (Christensen et al., 2011). Microcystin concentrations were detected in 78% of bloom samples. Of these, 50% were above 1 µg/L, and two samples were above the high risk WHO recreational level of 20 µg/L.

A study from 2002 evaluated water quality including chlorophyll-a concentration, cyanobacterial assemblages, and microcystin concentrations in 11 potable water supply reservoirs within the North Carolina Piedmont during dry summer growing seasons (Touchette et al., 2007). Microcystins concentrations were assessed using ELISA. The study found that cyanobacteria were the dominant phytoplankton community, averaging 65-95% of the total phytoplankton cells. Although microcystin concentrations were detected in nearly all source water samples, concentrations were <0.8 µg/L.

Since 2007, Ohio EPA (OHEPA, 2012) has been monitoring inland lakes for cyanotoxins. Of the 19 lakes in Ohio sampled during the NLA, 36% had detectable levels of microcystins. In

2010, OHEPA sampled Grand Lake St. Marys for anatoxin-a, cylindrospermopsin, microcystins, and saxitoxin. Toxin levels ranged from below the detection limit (<0.15 µg/L) to more than 2,000 µg/L for microcystins. Follow-up samples taken in 2011 for microcystins indicated concentrations exceeding 50 µg/L in August. During the same month, sampling in Lake Erie found microcystins levels exceeding 100 µg/L.

In 2008, NOAA began monitoring for cyanobacterial blooms in Lake Erie using high temporal resolution satellite imagery. Between 2008 and 2010, *Microcystis* cyanobacterial blooms were detected associated with water temperatures above 18°C (Wynne et al., 2013). Using the Great Lakes Coastal Forecast System (GLCFS), forecasts of bloom transport are created to estimate the trajectory of the bloom, and these are distributed as bulletins to local managers, health departments, researchers and other stakeholders. To evaluate bloom toxicity, the Great Lakes Environmental Research Laboratory (GLERL) collected samples at six stations each week for 24 weeks, measuring toxin concentrations as well as chlorophyll biomass and an additional 18 parameters (e.g., nutrients) to improve future forecasts of these blooms. In 2014, particulate toxin concentrations, collected from 1 meter depth, ranged from below detection to 36.7 µg/L. Particulate toxin concentrations peaked in August, 2014 at all sites, with the Maumee Bay site yielding the highest toxin concentration of the entire sampling period. Dissolved toxin concentrations were collected at each site from September until November when the field season ended. During the final months of sampling (October-November), dissolved toxin concentrations were detected with peak concentrations of 0.8 µg/L (mean: 0.28 +/- 0.2 µg/L) whereas particulate toxin concentrations were below detection limits on many dates, indicating that a majority of the toxins (mean: 72% +/- 37%) were in the dissolved pool as the bloom declined in intensity.

Concentrations of microcystins were detected during sampling in 2005 and 2006 in lakes and ponds used as a source of drinking water within the Lake Ontario watershed (Makarewicz et al., 2009). A microcystin-LR concentration of 5.07 µg/L was found in Conesus Lake, a source of public water supply that provides drinking water to approximately 15,000 people. Microcystin-LR was also detected at 10.716 µg/L in Silver Lake, a public drinking water supply for four municipalities.

2.3.2 Occurrence in 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. Currently, there is no program in place to monitor for the occurrence of cyanotoxins at surface-water treatment plants for drinking water in the U.S. Therefore, data on the presence or absence of cyanotoxins in finished drinking water are limited.

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, microcystin was found in 80% (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 U.S. (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.

2.4 Environmental Fate

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

2.4.1 Persistence

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. Microcystin is not destroyed by boiling (Rao et al., 2002). In natural waters kept in the dark, microcystins have been observed to persist for 21 days to 2-3 months in solution and up to 6 months in dry scum (Rapala et al., 2006; Funari and Testai, 2008).

In the presence of full sunlight, microcystins undergo photochemical breakdown, but this varies by microcystin congener (WHO, 1999; Chorus et al., 2000). The presence of water-soluble 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., 1993; 1995). According to Tsuji et al, microcystin-LR was photodegraded with a half-life (time it takes half of the toxin to degrade) of about 5 days in the presence of 5 mg/L of extractable cyanobacterial pigment. Humic substances can also act as photosensitizers and can increase the rate of microcystin breakdown in sunlight. In deeper or turbid water, the breakdown rate is slower.

Microcystins are susceptible to degradation by aquatic bacteria found naturally in rivers and reservoirs (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 (Jones et al., 1994; Cousins et al., 1996; Lahti et al., 1997a), and lake sediment (Rapala

et al., 1994; Lahti et al., 1997b). Lam et al., in 1995 reported that the biotransformation of microcystin-LR followed a first-order decay with a half-life of 0.2 to 3.6 days (Lam et al., 1995). In a study done by Jones et al. (1994) with microcystin-LR in different natural surface waters, microcystin-LR persisted for 3 days to 3 weeks; however, more than 95% loss occurred within 3 to 4 days. A study by Christoffersen et al., 2002, measured half-lives in the laboratory and in the field of approximately 1 day, driven largely by bacterial aerobic metabolism. These researchers found that approximately 90% of the initial amount of microcystin disappeared from the water phase within 5 days, irrespective of the starting concentration. Other researchers (Edwards et al., 2008) have reported half-lives of 4 to 14 days, with longer half-lives associated with a flowing stream and shorter half-lives associated with lakes.

2.4.2 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 (Han et al., 2012; Falconer, 1998). Ground water is generally not expected to be at risk of cyanotoxin contamination, however, ground water under the direct influence of surface water can be vulnerable. A study conducted by the USGS and the University of Central Florida determined that microcystin and cylindrospermopsin did not sorb in sandy aquifers and were transported along with ground water (O'Reilly et al., 2011). The authors suggested that the removal of microcystin was due to biodegradation.

2.5 Nature of the Stressor-Characteristics of the Microcystin Toxins

2.5.1 Toxicokinetics

No data were available that quantified the intestinal, respiratory or dermal absorption of microcystins. Available data indicate that the Organic Acid Transporter polypeptide (OATp) receptors facilitate the absorption of toxins from the intestinal tract into liver, brain, and other tissues. The OATp family transporters facilitate the cellular, sodium-independent uptake and export of amphipathic compounds such as bile salts, steroids, drugs, peptides and toxins (Cheng et al., 2005; Fischer et al., 2005; Svoboda et al., 2011). This facilitated transport is necessary for both uptake of microcystins into organs and tissues as well as for their export. Microcystins compete with bile acids for uptake by the liver and is limited in the presence of bile acids and other physiologically-relevant substrates for the transporter (Thompson and Pace, 1992). Other studies following *in vitro* or *in vivo* exposures have shown that inhibition of microcystin uptake by its OATp transporter reduces or eliminates the liver toxicity observed (Runnegar et al., 1981, 1995; Runnegar and Falconer, 1982; Hermansky et al., 1990a, b).

Limited information is available on the metabolism of microcystins. Some studies have found that metabolism of microcystin-LR in mice occurs in the liver (Robinson et al., 1991; Pace et al., 1991). Most of the available studies show minimal if any catabolism (process of breaking down molecules into smaller units to release energy). Microcystins can be conjugated with cysteine and glutathione to increase their solubility and facilitate excretion (Kondo et al., 1996).

However, it is not clear whether hepatic cytochromes, such as cytochrome P450-facilitated oxidation, precedes conjugation (Cote et al., 1986; Brooks and Codd, 1987). Both *in vivo* and *in vitro* studies have shown biliary excretion (Falconer et al., 1986; Pace et al., 1991; Robinson et al., 1991).

2.5.2 Noncancer Health Effects Data

2.5.2.1 Human Studies

The human data on the oral toxicity of microcystin-LR are limited by the potential co-exposure to other pathogens and toxins, by the lack of quantitative information, and by the failure to control for confounding factors.

Only a few epidemiological and case studies are available on the toxicity of microcystins in humans. An outbreak among army recruits who had consumed reservoir water with a cyanobacteria bloom with *M. 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 *Escherichia 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.

An epidemiology study done in Australia compared the hepatic enzyme levels from patients served by a public water supply contaminated with a *M. 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 done in China assessed the relationship between the consumption of water and food (carp and duck) contaminated with microcystins and liver damage in children (Li et al., 2011a). 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 microcystin exposure.

Acute intoxication with microcystin-producing cyanobacteria blooms in recreational water was reported in Argentina in 2007 (Giannuzzi et al., 2011). A single person was immersed in a *Microcystis* bloom with concentrations of 48.6 µg/L. After four hours of exposure, the patient exhibited fever, nausea, and abdominal pain, and three days later, presented dyspnea and respiratory distress and was diagnosed with an atypical pneumonia. A week after the exposure, the patient developed a hepatotoxicosis with a significant increase of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ -glutamyltransferase (γ GT). The patient completely recovered within 20 days.

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. 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).

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 cholestasis (elevations of AST, ALT bilirubin, ALP and GGT) in serum during weeks one to eight after treatment frequently exceeded normal values. Since microcystin-LR was not detected 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.

2.5.2.2 Animal Studies

Most of the information on the noncancer effects of microcystins in animals is from oral and intraperitoneal (i.p.) administration studies in mice and rats exposed to purified microcystin-LR. Liver effects are observed following acute oral exposure to microcystin-LR (Yoshida et al., 1997; Ito et al., 1997b; Fawell et al., 1999). Effects on the liver, kidney, and male reproductive system (testicular function and sperm quality), including changes in organ weights and histopathological lesions, are observed following short-term and subchronic oral exposure to microcystin-LR (Heinze, 1999; Fawell et al., 1999; Huang et al., 2011; Chen et al., 2011). Oral and i.p. developmental toxicity studies in mice provide some evidence for fetal body weight changes and maternal mortality (Fawell et al., 1999; Chernoff et al., 2002).

According to the authors, no clinical signs of toxicity were observed in a chronic study done in mice for 18 months by Ueno et al. (1999). Although histopathology from a 280 day study in mice revealed infiltrating lymphocytes and fatty degeneration in the livers, no quantitative data were provided in the study (Zhang et al., 2012).

2.5.3 Mode of Action for Noncancer Health Effects

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

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

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

2.5.4 Carcinogenicity Data

Several human epidemiological studies from China have reported an association between liver or colon cancer and consumption of drinking water from surface waters containing cyanobacteria and microcystins (Ueno et al., 1996; Zhou et al., 2002). In these studies, a concentrations measured in a surface drinking water supply were used as a surrogate for exposure to microcystins. Individual exposure to microcystins was not estimated, and there was no examination of numerous possible confounding factors, such as co-occurring chemical contaminants or hepatitis infections in the population.

A study done by Flemming et al. (2002, 2004) in Florida failed to find a significant association for primary liver cancer between populations living in areas receiving their drinking water from a surface water treatment plant (with the potential for microcystin exposures), and the Florida general population, or those receiving their water from ground-water sources. The one significant association observed was between those people in the surface water service areas, versus those in their surrounding areas described as buffer zones. However, the nature of the water supply for the buffer zones were not identified.

The only longer-term oral animal study of purified microcystin-LR was conducted by Ito et al. (1997b). Ito et al. (1997b) administered 80 µg microcystin-LR/kg/day by gavage to mice for 80 or 100 days over 28 weeks (7 months). This single dose failed to induce neoplastic nodules of the liver. The lack of hyperplastic nodules at 7 months suggests that microcystins are not a mutagenic initiator of tumors, however, the fairly short duration may have been a limiting factor.

Several studies suggest that microcystin-LR is a tumor promoter. In these studies, animals were first exposed to substances known to be tumor initiators (e.g. N-methyl-N-nitroso urea or NDEA) alone, or in combination with microcystin-LR at i.p. doses known to have no significant impact on liver weight. The combination of the initiator and the microcystin-LR significantly increased the number and area of glutathione *S*-transferase placental form-positive (GST-P) foci when compared to treatment with the initiator alone. The same was true for situations where the initiator treatment was combined with a partial hepatectomy (to stimulate tissue repair) and then exposed to microcystins i.p. (Nishiwaki-Matsushima et al., 1992; Ohta et al., 1994). GST-P foci are regarded as indicators for potential tumors formation. The results from these studies support the classification of microcystin as a tumor promoter.

2.6 Conceptual Model for Microcystins

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 HA values. The conceptual model demonstrates the relationship between exposure to microcystins in drinking water and adverse health effects in the populations at risk.

HAs describe non-regulatory concentrations of drinking water contaminants at which adverse health effects are not anticipated to occur over specific exposure durations (e.g., one-day, ten-days, and a lifetime). HAs also contain a margin of safety to protect sensitive members of the population. They serve as informal technical guidance to assist federal, state and local officials, as well as managers of public or community water systems, in protecting public health. They are not to be construed as legally enforceable federal standards.

Assessment endpoints for HAs can be developed for both short-term (one-day and ten-day) and lifetime exposure periods using information on the non-carcinogenic and carcinogenic toxicological endpoints of concern. Where data are available, endpoints will reflect susceptible and/or more highly exposed populations.

- A One-day HA is typically calculated for an infant (0-12 months or 10kg child), assuming a single acute exposure to the chemical and is generally derived from a study of less than seven days' duration.
- A Ten-day HA is typically calculated for an infant (0-12 months or 10kg child), assuming a limited period of exposure of one to two weeks, and is generally derived from a study of 7 to 30-days duration.

- A Lifetime HA is derived for an adult (>21 years or 80kg adult), and assumes an exposure period over a lifetime (approximately 70 years). It is usually derived from a chronic study of two years duration, but subchronic studies may be used by adjusting the uncertainty factor employed in the calculation. For carcinogens, the HA documents typically provide the concentrations in drinking water associated with risks for one excess cancer case per ten thousand persons exposed up to one excess cancer case per million exposed for Group A and B carcinogens and those classified as known or likely carcinogens (U.S. EPA, 1986, 2005). Cancer risks are not provided for Group C carcinogens or those classified as “suggestive”, unless the cancer risk has been quantified.

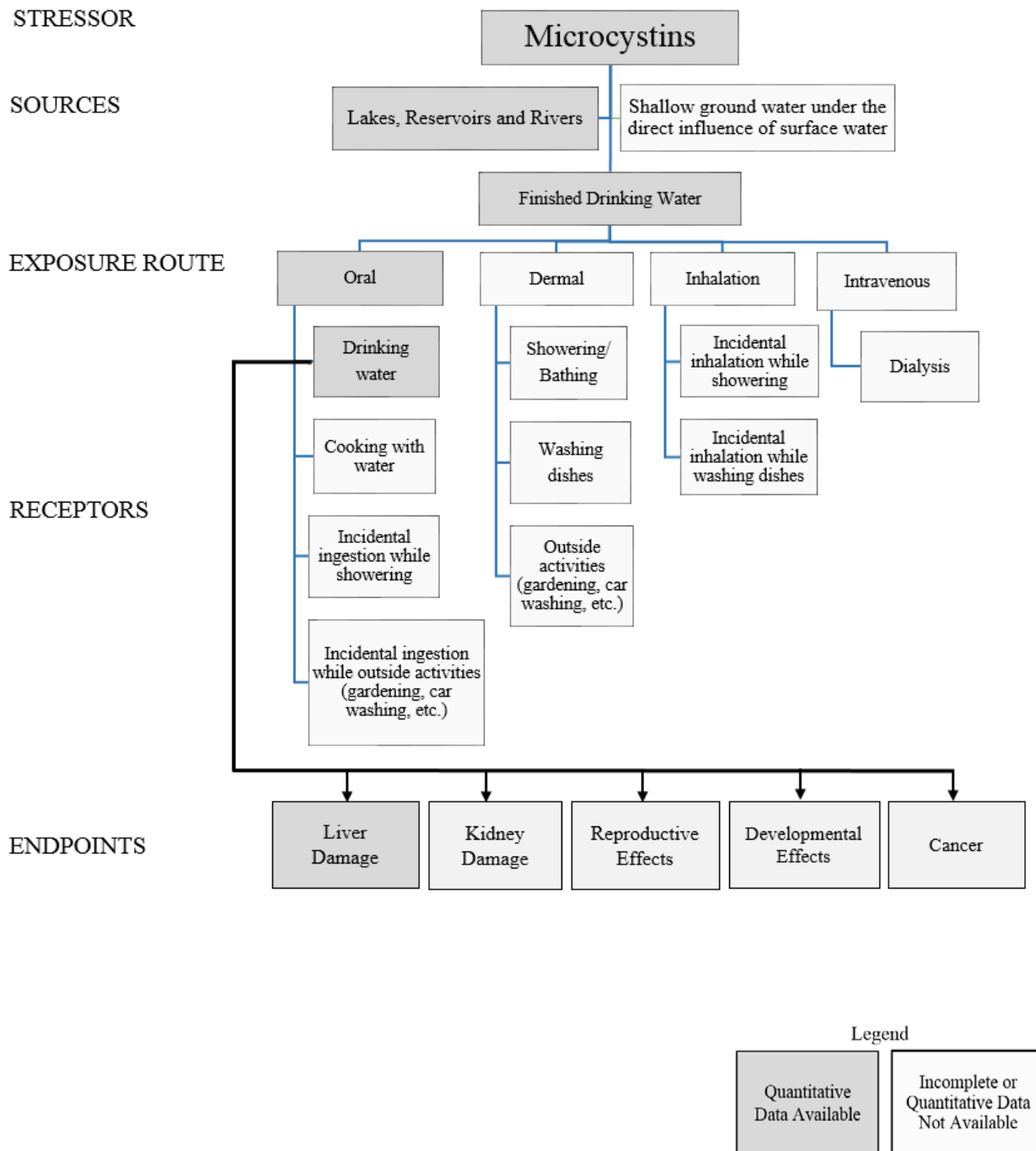
For each assessment endpoint EPA uses one or more measures of effect (also referred to as a point of departure), which describe the change in the attribute of the assessment endpoint in response to chemical exposure, to develop acute, short-term, longer term (subchronic) or chronic reference values when the data are available. The measures of effect selected represent impacts on survival, growth, system function, reproduction and development.

This conceptual model provides useful information to characterize and communicate the potential health risks related to exposure to cyanotoxins in drinking water. The sources of cyanotoxins in drinking water, the route of exposure for biological receptors of concern (e.g., via various human activities such as drinking, food preparation and consumption) and the potential assessment endpoints (i.e., effects such as kidney and liver toxicity, and reproductive and developmental effects) due to exposure to microcystins are depicted in the conceptual diagram below (Figure 2-3).

2.6.1 Conceptual Model Diagram

Cyanobacteria are a common part of freshwater and marine ecosystems. An increase in water column stability, high water temperatures, elevated concentrations of nutrients, and low light intensity have been associated with an increase and or dominance of microcystin-producing cyanobacteria in surface waters (or aquatic ecosystems). The presence of detectable concentrations of cyanotoxins in the environment is closely associated with these blooms. Winds and water currents can potentially transport cyanobacterial blooms to areas within the proximity of water intakes for drinking water treatment plants. If not managed in source waters, or removed during drinking water treatment, cyanobacteria and cyanotoxins may result in exposure that could potentially affect human health.

Figure 2-3. Conceptual Model of Exposure Pathways to Microcystins in Drinking Water



2.6.2 Factors Considered in the Conceptual Model for Microcystins

Stressors: For this HA, the stressor is microcystins concentrations in finished drinking water.

Sources: Sources of microcystins include potential sources of drinking water such as rivers, reservoirs, and lakes in the U.S. where blooms producing microcystins occur. Shallow private wells under the direct influence of surface water (in hydraulic connection to a surface water body) can also be impacted by microcystins-producing blooms, if the toxins are drawn into the well along with the water from the surface water. There is substantially less information on exposure from this source.

Routes of exposure: Exposure to cyanotoxins from contaminated drinking water sources may occur via oral exposure (drinking water, cooking with water, and incidental ingesting from showering); dermal exposure (contact of exposed parts of the body during bathing or showering, washing dishes, or outside activities); inhalation exposure (during bathing, showering or washing dishes.); or intravenous exposure (e.g. via dialysis). Toxicity data are available for the oral route of exposure from drinking water, but are not available to quantify dose response for other exposure routes (inhalation, dermal, dietary, and intravenous exposures).

Receptors: The general population (adults and children) could be exposed to cyanotoxins through dermal contact, inhalation and/or ingestion. Infants and pre-school age children can be at greater risk to microcystins because they consume more water per body weight than do adults. Other individuals of potential sensitivity are persons with kidney and/or liver disease due to the compromised detoxification mechanisms in the liver and impaired excretory mechanisms in the kidney. There are no human data to quantify risk to pregnant woman or to evaluate the transfer of cyanotoxins across the placenta. Data are also not available on the transfer of cyanotoxins through the milk from nursing mothers or regarding the risk to the elderly. Given this lack of information, pregnant women, nursing mothers, and the elderly may also be potentially sensitive populations. Data from the episode in a dialysis clinic in Caruaru, Brazil where microcystins were not removed by treatment of dialysis water, identify dialysis patients as a population of potential concern in cases where the drinking water source for the clinic is contaminated with cyanotoxins. Data are not available to derive a One-day HA for children because studies with single oral dosing do not provide dose-response information. A lifetime HA for microcystins is not recommended as the types of exposures being considered are short-term and episodic in nature. Although the majority of the cyanobacterial blooms in the U.S. occur seasonally, usually during late summer, some toxin-producing strains can occur early in the season and can last for days or weeks.

Endpoints: Human data on oral toxicity of microcystins are limited, but suggest the liver as the primary target organ. Acute, short-term, and subchronic studies in animals also demonstrate that the liver and kidney are target organs. In addition, some studies suggest that microcystins may lead to reproductive and developmental effects. Studies have suggested that microcystins have tumor promotion potential if there has been co-exposure to a carcinogen or cellular organ damage. However, these data are limited, and there has been no long term bioassay in animals to evaluate cancer. Available toxicity data are described in the *Health Effects Support Document (HESD) for Microcystins* (U.S. EPA, 2015a), and indicate that the primary target organ for microcystins is the liver. Kidney and reproductive effects in male mice were also observed, but were either not as

sensitive as the liver or lack confirmation from more than one laboratory. Data are inadequate to assess the carcinogenic potential of microcystins at this time.

2.7 Analysis Plan

The *Health Effects Support Document for Microcystins* (HESD, U.S. EPA, 2015a), provides the health effects basis for development of the HA, including the science-based decisions providing the basis for estimating the point of departure. To develop the HESD for microcystins, a comprehensive literature search was conducted from January 2013 to May 2014 using Toxicology Literature Online (TOXLINE), PubMed component, and Google Scholar to ensure the most recent published information on microcystins was included. Some of the search terms included in the literature search were microcystin, microcystin congeners, human toxicity, animal toxicity, *in vitro* toxicity, *in vivo* toxicity, occurrence, environmental fate, mobility, and persistence. EPA assembled available information on occurrence, environmental fate, mechanisms of toxicity, acute, short-term, subchronic and chronic toxicity and cancer in humans and animals, toxicokinetics, and exposure. Additionally, EPA considered information from the following risk assessments during the development of the microcystins health risk assessment:

- Health Canada (2012) *Toxicity Profile for Cyanobacterial Toxins*
- Enzo Funari and Emanuela Testai (2008) *Human Health Risk Assessment Related to Cyanotoxins Exposure*
- Tai Nguyen Duy, Paul Lam, Glen Shaw and Des Connell (2000) *Toxicology and Risk Assessment of Freshwater Cyanobacterial (Blue-Green Algal) Toxins in Water*

The toxicity data available for an individual pollutant vary significantly. An evaluation of available data was performed by EPA to determine data acceptability. The following study quality considerations from U.S. EPA's (2002) *A Review of the Reference Dose and Reference Concentration Processes* were used in selection of the studies for inclusion in the HESD and development of the HA.

- Clearly defined and stated hypothesis.
- Adequate description of the study protocol, methods, and statistical analyses.
- Evaluation of appropriate endpoints. Toxicity depends on the amount, duration, timing, and pattern of exposure and may range from frank effects (e.g., mortality) to more subtle biochemical, physiological, pathological, or functional changes in multiple organs and tissues.
- Application of the appropriate statistical procedures to determine an effect.
- Establishment of dose-response relationship (i.e., no observed adverse effect level (NOAEL) and/or lowest observed adverse effect level (LOAEL) or data amenable to modeling of the dose-response in order to identify a point of departure for a change in the effect considered to be adverse (out of the range of normal biological viability). The NOAEL is the highest exposure level at which there are no biologically significant

increases in the frequency or severity of adverse effect between the exposed population and its appropriate control. The LOAEL is the lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

After the available studies were evaluated for inclusion in the HESD and HA, the critical study was selected based on consideration of factors including exposure duration (comparable to the duration of the HA being derived), route of exposure (oral exposure via drinking water, gavage, or diet is preferred), species sensitivity, comparison of the point of departure with other available studies demonstrating an effect, and confidence in the study (U.S. EPA, 1999). Once, a point of departure is chosen for quantification, uncertainty factors appropriate for the study selected are then applied to the point of departure to account for variability and uncertainty in the available data.

For microcystins, toxicity and exposure data are available to develop a Ten-day HA. EPA used measures of effect and estimates of exposure to derive the Ten-day HAs using the following equation:

$$HA = \frac{NOAEL \text{ or } LOAEL \text{ or } BMDL}{UF \times DWI/BW}$$

Where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (mg/kg bw/day) from a study of an appropriate duration (7 to 30 days).

BMDL = When the data available are adequate, benchmark dose (BMD) modeling can be performed to determine the point of departure for the calculation of HAs. The benchmark dose approach involves dose-response modeling to obtain dose levels corresponding to a specific response level near the low end of the observable range of the data (U.S.EPA, 2012). The lower 95% confidence limit is termed the benchmark dose level (BMDL).

UF = Uncertainty factors (UF) account for: (1) intraspecies variability (variation in susceptibility across individuals); (2) interspecies variability (uncertainty in extrapolating animal data to humans); (3) uncertainty in extrapolating from a LOAEL to a NOAEL; and (4) uncertainty associated with extrapolation when the database is incomplete. These are described in U.S. EPA, 1999 and U.S. EPA, 2002.

DWI/BW = For children, a normalized ratio of drinking water ingestion to body weight (DWI/BW) was calculated using data for infants (birth to <12 months). The estimated drinking water intake body weight ratio (L/kg/day) used for birth to

<12 months of age are the 90th percentile values of the consumers only estimates of direct and indirect water ingestion based on 1994-1996, 1998 CSFII (Continuing Survey of Food Intakes by Individuals) (community water, mL/kg/day) in Table 3-19 in the U.S. EPA (2011a) *Exposure Factors Handbook*. The time weighted average of DWI/BW ratios values was derived from multiplication of age-specific DWI/BW ratios (birth to <1 month, 1 to <3 months, 3 to <6 months, and 6 to <12 months) by the age-specific fraction of infant exposures for these time periods.

For adults (>21 years of age), EPA updated the default BW assumption to 80 kg based on National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2006 as reported in Table 8.1 of EPA's *Exposure Factors Handbook* (U.S. EPA, 2011a). The updated BW represents the mean weight for adults ages 21 and older.

EPA updated the default DWI to 2.5 L/d, rounded from 2.546 L/d, based on NHANES data from 2003 to 2006 as reported in EPA's *Exposure Factors Handbook* (U.S. EPA 2011a, Table 3-33). This rate represents the consumer's only estimate of combined direct and indirect community water ingestion at the 90th percentile for adults ages 21 and older.

3.0 HEALTH EFFECTS ASSESSMENT

The health effects assessment provides the characterization of adverse effects and includes the hazard identification and dose-response assessment. The hazard identification includes consideration of available information on toxicokinetics; identification, synthesis and evaluation of studies describing the health effects of microcystins; and the potential modes of action (MOAs), or toxicity pathways related to the health effects identified.

3.1 Dose-Response

3.1.1 Study Selection

The critical study chosen for determining the guideline value is a short-term study by Heinze (1999) in which 11-week-old male hybrid rats (F1 generation of female WELS/Fohm x male BDIX) were administered microcystin-LR via drinking water for 28 days at concentrations of 0 (n=10), 50 (n=10) or 150 (n=10) µg/kg body weight (Heinze, 1999). Water consumption was measured daily, and rats were weighed at weekly intervals. The dose estimates provided by the authors were not adjusted to account for incomplete drinking water consumption (3-7% of supplied water was not consumed over the 28-day period). Rats were sacrificed by exsanguination under ether anesthesia after 28 days of exposure, and evaluation of hematology, serum biochemistry plus histopathology of liver and kidneys, and measurement of organ weights (liver, kidneys, adrenals, spleen and thymus) was performed.

Hematological evaluation showed an increase of 38% in the number of leukocytes at the highest dose group (150 µg/kg body weight). Serum biochemistry showed a significant increase in both treatment groups in mean levels of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH); 84 and 100% increase in LDH, and 34 and 33% increase in ALP, in the low and high dose groups respectively. No changes were observed in mean levels of AST (aspartate aminotransferase), and ALT (alanine aminotransferase). An increase in relative liver weights was observed in a dose-dependent manner; 17% at 50 µg/kg body weight, and 26% at 150 µg/kg body weight. Mean enzyme levels and relative liver weights are shown in Table 3-1.

A dose-dependent increase in absolute liver weight was also reported, and data on the liver weights were provided by the author in a personal communication. A dose-dependent increase in the average absolute liver weights was also observed in all groups: 8.8 grams at the control group, 9.70 grams at the lower dose and 10.51 grams at the high dose (Table 3-1). No statistically significant changes in other organ weights or body weights were reported, and no effects on the kidneys were observed. Table 3-2 summarizes the histological observations of liver lesions. Liver lesions were considered toxic and spread diffusely throughout the parenchyma indicating cell damage expressed by an increase in cell volume, an increase in mitochondria, cell necrosis, the activation of Kupffer cells, and an increase in the amounts of periodic acid-Schiff (PAS)-positive substances. Liver lesions were observed in both treatment groups. No kidney effects were observed in either dose groups. The LOAEL was determined to be 50 µg/kg/day. The selection of Heinze (1999) as the critical study was based on the appropriateness of the study duration, the use

Table 3-1. Liver Weights and Serum Enzyme Levels in Rats Ingesting Microcystin-LR in Drinking Water (Heinze, 1999)

	Control (Mean ± SD)	50 µg/kg (Mean ± SD)	150 µg/kg (Mean ± SD)
Serum Enzymes			
Alkaline phosphatase (ALP) (microkatal/L)	9.67 ± 2.20	13.00 ± 3.81*	12.86 ± 1.85*
Lactate dehydrogenase (LDH) (microkatal/L)	16.64 ± 4.48	30.64 ± 5.05*	33.58 ± 1.16*
Liver Weight			
Relative (g/100 g body weight)	2.75 ± 0.29	3.22 ± 0.34*	3.47 ± 0.49*
Absolute (g)**	8.28 ± 1.37	9.70 ± 1.32	10.51 ± 1.02

* p≤0.05 when compared with control; katal=conversion rate of 1 mole of substrate per second.

**Information provided by the author through a personal communication.

Table 3-2. Histological Evaluation of the Rat Livers after Ingesting Microcystin-LR in Drinking Water (Heinze, 1999)

	Activation of Kupffer Cells	Degenerative and Necrotic Hepatocytes with Hemorrhage	Degenerative and Necrotic Hepatocytes without Hemorrhage	PAS-positive Material
Control				
Slight	0	0	0	1
Moderate	0	0	0	0
Intensive damage	0	0	0	0
50 µg/kg				
Slight	0	4	0	5
Moderate	10	6	0	5
Intensive damage	0	0	0	0
150 µg/kg				
Slight	0	0	0	0
Moderate	10	6	1	8
Intensive damage	0	3	0	2

of multiple doses, dose-related toxicological responses, and histopathological evaluations of toxicity.

3.1.2 Endpoint Selection

The point of departure selected from the Heinze (1999) study is the LOAEL (50 µg/kg/day) for liver effects (increased liver weight, slight to moderate liver necrosis lesions, with or without hemorrhages at the low dose and increased severity at the high dose, and changes in serum enzymes indicative of liver damage). For the lesions, incidence increases from one animal impacted in the control group to ten animals impacted in the dosed groups. This dose-response is more dramatic than the difference in liver weight between the control and low dose (1.17 fold) and the differences in the ALP and LDH levels between the control and low dose group (1.34 and 1.84-fold, respectively). Therefore, the liver lesions are identified as the endpoint of greatest concern. These differences also advise against application of benchmark dose modeling for these effects. The male and female mice in the Fawell et al (1999) study displayed liver lesions, but the difference between controls and the low dose group (40 µg/kg/day) was less than two-fold. In an i.p. infusion study by Guzman and Solter (1999) with a more direct delivery of dose to the liver, necrosis was observed at doses of 32 and 48 µg/kg/day, but not at a dose of 16 µg/kg/day, thus providing support for the critical effect and dose.

3.2 Ten-day Health Advisory

This Ten-day HA is applied to total microcystins using microcystin-LR as a surrogate. The Ten-day HA is considered protective of non-carcinogenic adverse health effects over a ten-day exposure to microcystins in drinking water.

3.2.1 Bottle-fed Infants and Young Children of Pre-school Age

The Ten-day HA for bottle-fed infants and young children of pre-school age is calculated as follows:

$$\text{Ten-day HA} = \frac{50 \text{ } \mu\text{g/kg/day}}{1000 \times 0.15 \text{ L/kg/day}} = 0.3 \text{ } \mu\text{g/L}$$

Where:

- 50 µg/kg/day = The LOAEL for liver effects in 11-week-old male hybrid rats exposed to microcystin-LR in drinking water for 28 days (Heinze, 1999).
- 1000 = The composite UF including a 10 for intraspecies variability (UF_H), a 10 for interspecies differences (UF_A), a 3 for LOAEL to NOAEL extrapolation (UF_L), and a 3 for uncertainties in the database (UF_D).
- 0.15 L/kg/day = Normalized drinking water intake per unit body weight over the first year of life based on the 90th percentile of drinking water consumption and the mean body weight (U.S. EPA, 2011a).

The Ten-day HA of 0.3 µg/L is considered protective of non-carcinogenic adverse health effects for bottle-fed infants and young children of pre-school age over a ten-day exposure to microcystins in drinking water.

3.2.2 School-age Children through Adults

The Ten-day HA for school-age children through adults is calculated as follows:

$$\text{Ten-day HA} = \frac{50 \text{ } \mu\text{g/kg/day}}{1000 \times 0.03 \text{ L/kg/day}} = 1.6 \text{ } \mu\text{g/L}$$

Where:

- 50 µg/kg/day = The LOAEL for liver effects in 11-week-old male hybrid rats exposed to microcystin-LR in drinking water for 28 days (Heinze, 1999).
- 1000 = The composite UF including a 10 for intraspecies variability (UF_H), a 10 for interspecies differences (UF_A), a 3 for LOAEL to NOAEL extrapolation (UF_L), and a 3 for uncertainties in the database (UF_D).
- 0.03 L/kg/day = Drinking water intake per unit body weight based on adult default values of 2.5 L/day and 80 kg (U.S. EPA, 2011a).

The Ten-day HA of 1.6 µg/L is considered protective of non-carcinogenic adverse health effects for children of school age through adults over a ten-day exposure to microcystins in drinking water.

3.2.3 Uncertainty Factor Application

- UF_H - A Ten-fold value is applied to account for variability in the human population. No information was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics among humans. Individuals with pre-existing liver problems could be more sensitive to microcystins exposures than the general population. Pregnant woman, nursing mothers, and the elderly could also be sensitive to microcystins exposures.
- UF_A - A Ten-fold value is applied to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). Information to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans is unavailable for microcystins. Allometric scaling is not applied in the development of the Ten-day HA values for microcystins. The allometric scaling approach is derived from the relationship between body surface area and basal metabolic rate in adults (U.S. EPA, 2011b). This approach is not appropriate for infants and children due to the comparatively slower clearance during these ages and the limited toxicokinetic data available to assess the appropriateness of body weight scaling in early life.

- UF_L - An uncertainty factor of 3 ($10^{0.5} = 3.16$) is selected to account for the extrapolation from a LOAEL to a NOAEL. The threefold factor is justified based on the evidence suggesting that the uptake of microcystins by tissues requires membrane transporters. Uptake from the intestines involves both apical and basolateral transporters, uptake by the microvilli capillaries and portal transport to the liver. Transporters are again necessary for hepatic uptake. When there is slow infusion into the peritoneum and into the portal intraperitoneal capillaries, uptake is described as rapid because of the rich blood supply and large surface area of the peritoneal cavity (Klassen, 1996). Delivery of the microcystins to the intraperitoneum increases the amount of the dose that reaches the liver for three additional reasons: 1) the apical and basolateral intestinal barriers to uptake are eliminated with the i.p. infusion; 2) there is no dilution of dose by the gastric plus intestinal fluids as when food residues are in the gastrointestinal track; and 3) there is no delay in reaching the site of absorption because of gastric emptying time (Klassen, 1996). In addition, facilitated transporter kinetics are similar to Michaelis Menton enzyme kinetics in that there are K_m and V_{max} components that are defined by the affinity of the transported substance for the transporter.

In the Guzman and Solter (1999) intraperitoneal infusion study in rats, the NOAEL is 16 $\mu\text{g}/\text{kg}/\text{day}$ and the LOAEL is 32 $\mu\text{g}/\text{kg}/\text{day}$, a two-fold difference. There is no reason to believe that the less direct delivery from the intestines to the liver following oral exposures through drinking water (as was used in Heinze, 1999) would have a more than 3-fold separation between a NOAEL and LOAEL had there been one in the Heinze (1999) study.

- UF_D - An uncertainty factor of 3 ($10^{0.5} = 3.16$) is selected to account for deficiencies in the database for microcystins. The database includes limited human data, including studies evaluating the association between microcystin exposure and cancers in liver and colon, and systemic effects including liver endpoints such as elevated liver enzymes. Oral and i.p. acute and short-term studies on mice and rats, and subchronic studies done in mice are available. Chronic data are also available for microcystin, however, are limited by the lack of quantitative data provided in the study. Additionally, there are limited neurotoxicity studies (including a recent publication on developmental neurotoxicity) and several i.p. reproductive and developmental toxicity studies. The database lacks a multi-generation reproductive toxicity study.

The default factors typically used cover a single order of magnitude (i.e., 10^1). By convention, in the Agency, a value of 3 is used in place of one-half power (i.e., $10^{1/2}$) when appropriate (U.S. EPA, 2002).

4.0 RISK CHARACTERIZATION

The following topics describe important conclusions used in the derivation of the health advisory. This section characterizes each topic and its impact on the health advisory.

4.1 Use of microcystin-LR as a surrogate for total microcystins

Among the approximately 100 different congeners of microcystins known to exist, microcystin-LR is the most common. The difference in toxicity of microcystin congeners depends on the amino acid composition (Falconer, 2005). Stoner et al. (1989) administered by intraperitoneal (i.p.) purified microcystin congeners (-LR, -LA, -LY and -RR) into ten or more adult male and female Swiss albino mice. Necropsies were performed to confirm the presence of the pathognomonic hemorrhagic livers. The authors reported 50% lethal doses (LD₅₀) of 36 ng/g-bw for -LR, 39 ng/g-bw for -LA, 91 ng/g-bw for -LY and 111 ng/g-bw for -RR. Similarly, Gupta et al., (2003) determined LD₅₀ for the microcystin congeners LR, RR and YR in female mice using DNA fragmentation assay and histopathology examinations of the liver and lung. The acute LD₅₀ determination showed that the most toxic congener was microcystin-LR (43.0 µg/kg), followed by microcystin-YR (110.6 µg/kg) and microcystin-RR (235.4 µg/kg). The most toxic microcystins are those with the more hydrophobic L-amino acids (-LA, -LR, -and -YM), and the least toxic are those with hydrophilic amino acids, such as microcystin-RR.

Wolf and Frank (2002) proposed toxicity equivalency factors (TEFs) for the four major microcystin congeners based on LD₅₀ values obtained after i.p. administration. The proposed TEFs, using microcystin-LR as the index compound (TEF=1.0) were 1.0 for microcystin-LA and microcystin-YR and 0.1 for microcystin-RR. The application of TEFs based on i.p. LD₅₀ values to assessment of risk from oral or dermal exposure is questionable given that differences in lipophilicity and polarity of the congeners may lead to variable absorption by non-injection routes of exposure.

The potential health risks from exposure to mixtures of microcystin congeners is unknown, and since microcystin-LR is one of the most potent congeners and has the majority of toxicological data on adverse health effects, microcystin-LR is used as a surrogate for all microcystins in the health advisory.

4.2 Consideration of Study Duration

EPA used a 28-day study conducted by Heinze (1999) to derive the Ten-day HA for microcystins. It is standard to use studies that are 7 to 30 days in duration to derive a 10-day advisory value. In the study conducted by Heinze (1999), rats were dosed daily via drinking water with microcystin and sacrificed at the conclusion of the study. No interim sacrifices were performed to evaluate effects at 10 days or any other time less than the full 28 days. At the conclusion of the 28-day study, adverse effects observed in the liver included increases in liver weight, slight to moderate liver necrosis lesions accompanying hemorrhages at the low dose with increased severity at the high dose, and changes in serum enzymes indicative of liver damage. Given the lack of interim effects data, it is not known when during the 28-day study these effects were manifested.

4.3 Consideration of Reproductive Effects as Endpoint

Upon consideration of all available studies, liver effects were considered the most appropriate basis for quantitation as it was a common finding among oral toxicology studies (Falconer et al., 1994; Fawell et al., 1999; Ito et al., 1997b). However, while the liver is the primary target of microcystin toxicity, there have been reports of effects of microcystin-LR on the male reproductive system and sperm development following oral exposures (Chen et al., 2011).

In a study conducted by Chen et al. (2011), oral exposures to low concentrations of microcystin-LR for 3 to 6 months showed reproductive toxicity including decreased sperm counts and sperm motility, as well as an increase in sperm abnormalities, decreased serum testosterone and increased serum luteinizing hormone (LH) levels. Because these effects were observed at doses lower (0.79 µg/kg/day) than those observed for liver effects in Heinze (1999), EPA evaluated Chen et al. (2011) and the lesions in the testes and effects on sperm motility as the potential critical study and points of departure for the derivation of the RfD for microcystins.

The Chen et al., 2011 study has several limitations in the experimental design and reporting. There was a lack of data reported on testis weights and sperm motility. The authors reported “no significant differences in testis weights,” but no information was provided on the weights of the testis or whether there was a trend toward decreasing weights that failed to be statistically significant. Also, no information was given on the methodology used for sperm motility evaluation. No information was provided on how samples were handled and what measurements were made to determine the percentage of sperm motility. Although body weight and amount of water consumed were measured, these data were not presented, and doses to the animals were not calculated by the study authors. In addition, the purity of microcystin-LR and the species and age of the mouse used were not reported. Male sperm characteristics such as volume, motility, and structure of sperm differ developmentally by age. Therefore, not knowing the age of the mice in the study introduces uncertainty in the quantification of the reproductive effects.

The fixation and staining of the testes used for microscopic examination (paraformaldehyde in phosphate-buffered saline (PBS) and paraffin), could result in the generation of artifacts, such as disruption of the testicular tubes. Cytoplasmic shrinkage and chromatin aggregations were observed in both control and experimental groups. In order to preserve the microstructure of the testis, dual fixation such as Davidson’s or Bouin’s fixation followed by PAS staining should have been done. In addition, the histopathology analysis of the testes reported by the authors did not provide sufficient detail to adequately assess the degree of damage.

The quality of the medium used for the sperm analysis, and the lack of additional data from the sperm analysis measurements carried out through the computer-assisted sperm analysis (CASA) are additional limitations in experimental design for this study. Very few details of the serum hormone assay protocol and the quantitative parameters of sperm motility from the CASA analysis were provided. Therefore, the calculation for the motility of the sperm was unclear and could not be verified.

Based on the limitations in study design, report and methods used by Chen et al. (2011), EPA concluded that the quantitative data on decreased sperm counts and sperm motility were not appropriate for determining the point of departure for the derivation of the RfD for microcystin.

4.4 Allometric Scaling Approach

Allometric scaling was not applied in the development of the short term RfD for microcystins. In the development of short-term advisory values (One-day and Ten-day), parameters are used that reflect exposures and effects for infants up to one year of age, rather than for adults. The body weight scaling approach is derived from the relationship between body surface area and basal metabolic rate in adults. Infants/children surface area and basal metabolic rates are very different than adults with a slower metabolic rate. In addition, limited toxicokinetic data are available to assess the appropriateness of body weight scaling in early life. The body weight scaling procedure has typically been applied in the derivation of chronic oral RfDs and cancer assessments, both of which are concerned with lifetime repeated exposure scenarios (U.S. EPA, 2012). Thus, given the short term duration of the critical study and the development of a short term RfD for determination of a Ten-day HA value, and the application of the Ten-day HA to infants and pre-school age children, the application of the body weight scaling procedure is not appropriate for this scenario.

In addition, for short-term advisories (one-day and ten-day duration), EPA assumes all exposure is derived from drinking water and, therefore, no Relative Source Contribution (RSC) term is applied. For lifetime health advisory values, EPA does include an RSC that reduces the advisory value to account for other potential sources.

4.5 Benchmark Dose (BMD) Modeling Analysis

The data set reported by Heinze (1999) was evaluated for BMD modeling. Heinze (1999) demonstrated dose-related liver changes and statistically significant effects at the lowest dose (50 $\mu\text{g}/\text{kg}/\text{day}$). Histological changes were also observed in all the animals (ten) in each dose group (Table 3-2). Although differences in the degree of necrosis were observed with or without hemorrhage related to dose, all the histological effects including Kupffer cell activation and PAS staining showed no dose-response since all ten animals at the low and high doses displayed liver damage associated with each effect. Therefore, the dose-response for the sum of the incidence categories (slight, moderate, and intensive damage), are not amenable to BMD modeling. As a result, the LOAEL of 50 $\mu\text{g}/\text{kg}/\text{day}$ described by Heinze (1999) was used as the POD for development of the HA.

4.6 Carcinogenicity Evaluation

While there is evidence of an association between liver and colorectal cancers in humans and microcystins exposure and some evidence that microcystin-LR is a tumor promoter in mechanistic studies, there is inadequate information to assess carcinogenic potential of microcystins in humans (U.S. EPA, 2005). The human studies are limited by lack of exposure

information and the uncertainty regarding whether or not these studies adequately controlled for confounding factors such as Hepatitis B infection. No chronic cancer bioassays for microcystins in animals are available.

The only oral study that examined the tumorigenicity of microcystin-LR failed to find preneoplastic nodules in the livers of groups of 22 mice receiving up to 100 doses of 0 or 80 $\mu\text{g}/\text{kg}/\text{day}$ over 7 months. Some studies suggest that microcystin-LR is a tumor promoter. Given the potential impact on the cell cytoskeleton, necrotic effects on liver cell generation of reactive oxygen species (ROS), and other biochemical changes, this finding is not surprising. The work by Nishiwaki-Matsushima et al., 1992 that compares glutathione *S*-transferase placental form-positive (P-GST) foci from 10 $\mu\text{g}/\text{L}$ microcystin-LR to that from the phenobarbital (0.05% in the diet) as a positive control suggests that it is at best a weak promoter. The results from the second part of the same study that compare P-GST foci following initiation with DEN followed by microcystin-LR (10 $\mu\text{g}/\text{kg}$), both before and after a partial hepatectomy, support this conclusion.

The International Agency for Research on Cancer (IARC) classified microcystin-LR as a Group 2B (possibly carcinogenic to humans) based on the conclusion that there was strong evidence supporting a plausible tumor promoter mechanism for these liver toxins. U.S. EPA's Cancer Guidelines (2005) state that the descriptor of "*inadequate information to assess carcinogenic potential*" is appropriate when available data are judged inadequate for applying one of the other descriptors or for situations where there is little or no pertinent information or conflicting information. The guidelines also state that (p. 2-52) "Descriptors can be selected for an agent that has not been tested in a cancer bioassay if sufficient other information, e.g., toxicokinetic and mode of action information, is available to make a strong, convincing, and logical case through scientific inference". In the case of microcystins, the data suggest that microcystin-LR may be a tumor promoter but not an initiator. Without stronger epidemiology data and a chronic bioassay of purified microcystin-LR, the data do not support classifying microcystin-LR as a carcinogen.

4.7 Uncertainty and Variability

Several uncertainty factors were applied in several areas to account for incomplete information. Human data on the toxic effects of microcystins are limited. Quantification of the absorption, distribution, and elimination of microcystins in humans following oral, inhalation or dermal exposure is not well understood. The clinical significance in humans for biological changes observed in experimental animals such as decreased sperm count and motility, and microscopic lesions in the testes needs further analysis. In animal studies with oral exposures to microcystins, some adverse effects in males such as reduced testosterone levels, as well as toxicity to the female reproductive tissues and those of offspring have not been fully characterized. No data are available to quantify the differences between humans and animals for the critical health endpoints. There is uncertainty regarding susceptibility and variability in the human population following exposure to microcystins and the relative toxicity of other microcystins congeners when compared to microcystin-LR. Additional information is needed on the potential health risks from mixtures of microcystins with other cyanotoxins, as well as biological and chemical stressors present in source water and drinking water supplies.

In addition, for short-term advisories (One-day and Ten-day duration), EPA assumes all exposure is derived from drinking water and, therefore, no Relative Source Contribution (RSC) term is applied. For lifetime health advisory values, EPA does include an RSC that reduces the advisory value to account for exposure to other potential sources.

4.8 Susceptibility

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 (ALT and AST), but no sex-related differences in liver pathology.

Studies in laboratory rodents suggest that the acute effects of microcystin-LR may be more pronounced in adult or aged animals than in juvenile animals (Adams et al., 1985; Ito et al., 1997a; Rao et al., 2005). In these studies, young animals showed little or no effect at microcystin-LR doses found to be lethal to adult animals. Age-dependent differences in toxicity were observed after both oral and i.p. exposure, suggesting that differences in gastrointestinal uptake were not entirely responsible for the effect of age. The relevance of these age-related differences to acute toxicity in humans is unknown. However, for infants to one-year olds fed exclusively with powdered formula prepared with tap water, drinking water is the dominant route of exposure to cyanotoxins. There are significant differences in exposure between these life-stages that impact risk.

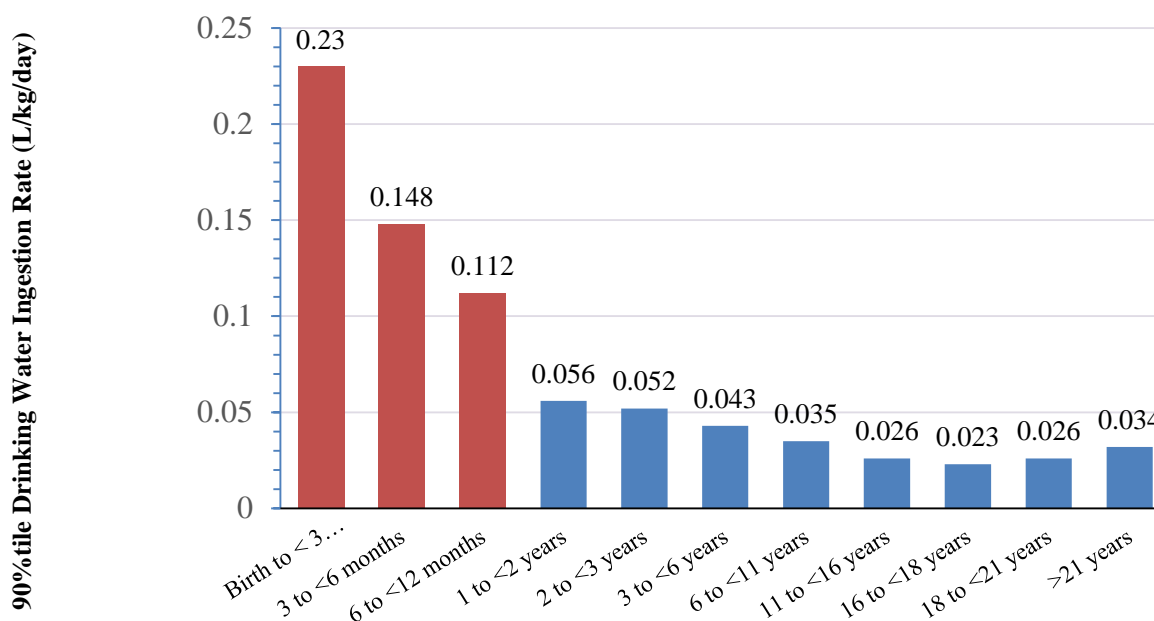
Based on the available studies in animals, individuals with liver and/or kidney disease may be more susceptible than the general population since the detoxification mechanisms in the liver and impaired excretory mechanisms in the kidney may be compromised. Data from an episode in a dialysis clinic in Caruaru, Brazil where microcystins were not removed by treatment of dialysis water, identify dialysis patients as a population of potential concern in cases where the drinking water source for the clinic used to prepare the dialysate is contaminated with cyanotoxins. Other potentially sensitive individuals include pregnant woman, nursing mothers, and the elderly.

4.9 Distribution of Body Weight and Drinking Water Intake by Age

Both body weight and drinking water intake are distributions that vary with age. EPA has developed two health advisory values, a Ten-day HA of 0.3 µg/L based on exposure to infants over the first year of life, and a Ten-day HA of 1.6 µg/L based on exposure to adults, over 21 years of age. Section 4.10 discusses how EPA recommends application of these values to other age groups.

The U.S. EPA (2011a) Exposure Factors Handbook provides values for drinking water ingestion rate and corresponding body weight. The estimated 90th percentile of community water ingestion for the general population (males and females of all ages) has been used as the default value for water ingestion. EPA plotted the 90th percentile of drinking water intake using Table 3-19 for ages ≤3 years, and Table 3-38 for ages >3 years due to sample size in the respective studies. Age groups <3 months in Table 3-19 were combined due to insufficient sample sizes. Figure 4.1

Figure 4-1. 90th Percentile Drinking Water Ingestion Rates by Age Group



Adapted from U.S. EPA 2011 Exposure Factors Handbook (U.S.EPA, 2011a).

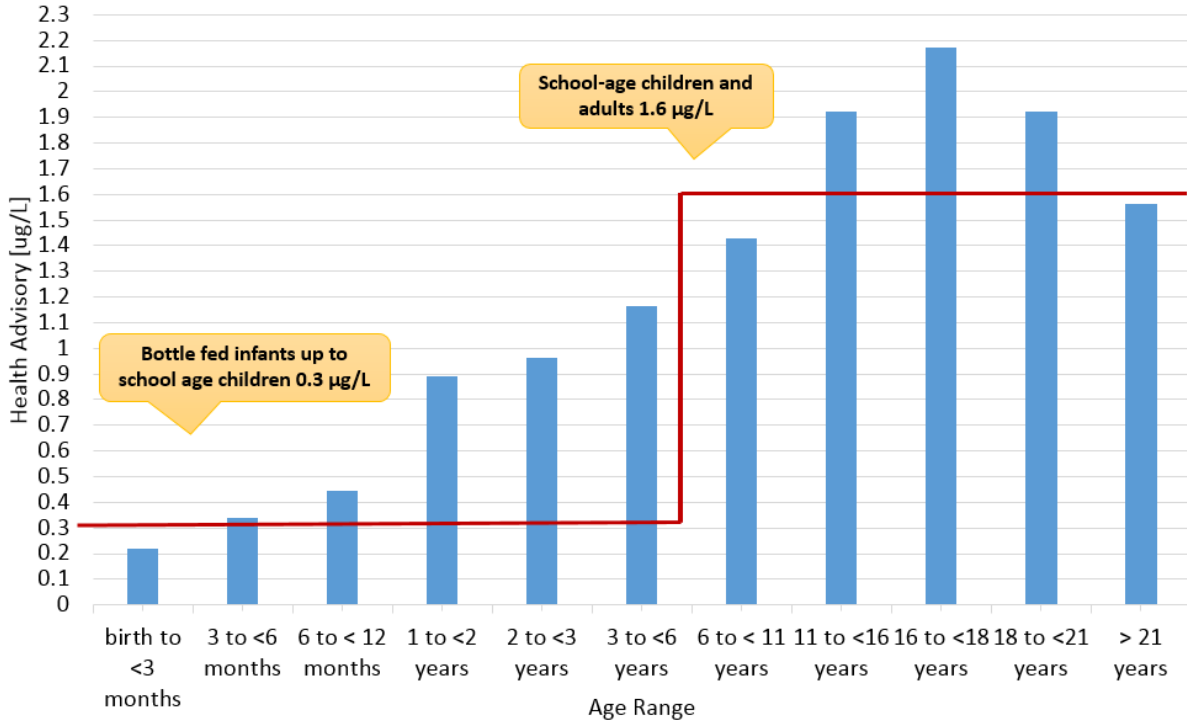
represents the 90th percentile drinking water ingestion rates (L/kg/day) for each age group (located on top of the columns). Bottle-fed ages are shown in red (first three columns on the left).

Based on the drinking water intake rates for children <12 months (0.15 L/kg-day), the exposure of children is over 4 times higher than that of adults >21 years old on a body weight basis (0.034 L/kg-day). Infants from birth to 3 months may be exclusively bottle-fed and therefore, have a higher ingestion rate. After 3 months of age, typically around 4 to 6 months of age, other food and liquids are introduced into the infant diet, lowering the ingestion rate of drinking water. Drinking water contributes the highest risk of the total cyanotoxin intake for infants to one-year-olds fed exclusively with powdered formula prepared with tap water containing cyanotoxins. At the age of 6, children’s intake of drinking water relative to their body weight is approximately the same as those of an adult (>21 years). Data evaluating the transfer of microcystins through breast milk are not available for humans.

4.10 Distribution of Potential Health Advisory Values by Age

Using the ingestion rates for each age-group (from Figure 4-1), EPA estimated Ten-day HA values for microcystins for each age group (plotted on Figure 4-2) to demonstrate the variability due to body weight and drinking water intake by age.

Figure 4-2. Ten-day Health Advisories for Microcystins by Age Group



EPA decided to apply the Ten-day HA value calculated for infants over the first year of life ($0.3 \mu\text{g/L}$) to all bottle-fed infants and young children of pre-school age because these age groups have higher intake per body weight relative to adults. As Figure 4.2 demonstrates, when the Ten-day HA is estimated by age group, the calculated HA value for infants from birth to 3 months old is $0.2 \mu\text{g/L}$, slightly below the infant health advisory value of $0.3 \mu\text{g/L}$. EPA believes that infants from birth to 3 months old are not at a disproportionate risk at a $0.3 \mu\text{g/L}$ advisory value because a 30-fold safety factor is built into this calculation to account for human variability and deficiencies in the database. The estimated Ten-day HA values for infants from 3 months old through pre-school age groups (less than 6 years old) are at or above the advisory value of $0.3 \mu\text{g/L}$. Therefore, children within these age groups are adequately protected by the advisory value for bottle-fed infants and young children of pre-school age. EPA decided to apply the adult Ten-day HA value of $1.6 \mu\text{g/L}$ to school age children (children older than or equal to 6 years) through adulthood because children's intake of drinking water relative to body weight in this age group is almost the same as those of an adult (>21 years).

5.0 ANALYTICAL METHODS

This Health Advisory (HA) for the Cyanobacterial Microcystin Toxins is applied to total microcystins which should include all of the measureable microcystin congeners within the cyanobacterial cells (intracellular) and outside the cell (extracellular).

Extracellular microcystins (either dissolved in water or bound to other materials) typically make up less than 30% of the total microcystin concentration in source water (Graham et al., 2010). Most of the toxin is intracellular, and released into the water when the cells rupture or die. Both intracellular and extracellular microcystins may also be present in treated water, depending on the type of treatment processes in place. Therefore, it is important to note that analysis for microcystins should account for both intracellular and extracellular toxins in samples when intact cells may be present. Release of intracellular microcystins is achieved by rupturing or lysing the cell walls in order to expose the intracellular microcystins. Cell lysis can be achieved by a variety of methods including sequential freeze-thawing, freeze drying, and mechanical or sonic homogenization. Following cell lysis, microcystins may need to be extracted for some analytical methods. At low concentrations, the direct determination of microcystins may not be feasible, and a preconcentration step may be required. Typically samples are filtered and/or centrifuged after cell lysis to remove cell fragments and particulates. This may be followed by freeze-drying or solid-phase extraction (SPE). Typical elution solvents are dilute acid, methanol, acidified methanol/water mixtures, and butanol/methanol/water mixtures.

Preconcentration is generally needed when techniques such as liquid chromatography are used in order to achieve limits of detection in the low- $\mu\text{g/L}$ and ng/L range. Extraction efficiency has been shown to vary depending on the type of solvent, the hydrophobicity of the congener, the water content of the cells (freeze-dried versus frozen) and differences between field samples and laboratory cultures. Variations in extraction efficiency may impact the accurate quantitation of microcystins so the use of a surrogate compound to monitor the extraction efficiency is strongly recommended. Responsible authorities should ensure that the appropriate methods and preparation techniques (extraction, concentration and separation) are being used in the laboratory depending on the type of sample and the analytical method selected.

Analytical methods available for the detection of microcystins in drinking water include reversed phase high performance liquid chromatography (HPLC) coupled with mass spectrometric (MS, MS/MS) or ultraviolet/photodiode array detectors (UV/PDA), Enzyme Linked Immunosorbent Assays (ELISA), and Protein Phosphatase Inhibition Assays (PPIA).

EPA has developed a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for microcystins and nodularin (combined intracellular and extracellular) in drinking water (Method 544; U.S. EPA, 2015). Accuracy and precision data have been generated in reagent water, and finished ground and surface waters for the following compounds: microcystin-LA (microcystin-LA), -LF (microcystin-LF), -LR (microcystin-LR), -LY (microcystin-LY), -RR (microcystin-RR), -YR (microcystin-YR), and nodularin-R (NOD). This method is intended for use by analysts skilled in solid phase extractions, operation of LC/MS/MS instruments, and the interpretation of associated data. The single laboratory lowest concentration minimum reporting levels (LCMRLs) for this method range from 2.9 to 22 ng/L (0.0029-0.022 $\mu\text{g/L}$). The Detection Limit (DL) for analytes in this method range from 1.2 to 4.6 ng/L . In this method, a 500 mL water

sample (fortified with an extraction surrogate) is filtered, and both the filtrate and the filter are collected. The filter is placed in a solution of methanol containing 20% reagent water and held for at least one hour at -20 °C to release the intracellular toxins from cyanobacteria cells captured on the filter. The liquid is drawn off the filter and added back to the 500-mL aqueous filtrate. The 500-mL sample (plus the intracellular toxin solution) is passed through a SPE cartridge to extract the method analytes and surrogate. Analytes are eluted from the solid phase with a small amount of methanol containing 10% reagent water. The extract is concentrated to dryness by evaporation with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with methanol containing 10% reagent water. A 10- μ L injection is made into an LC equipped with a C8 column that is interfaced to an MS/MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by external standard calibration. To download Method 544 Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/tandem Mass Spectrometry (LC/MS/MS), please go to: <http://www.epa.gov/nerlcwww/ordmeth.htm>

High performance liquid chromatography (HPLC) is widely used to separate microcystin congeners. A variety of stationary phases have been used including reversed-phase C₁₈ columns, amide C₁₈ columns, internal surface reversed-phase columns or ion exchange columns. Optimization of chromatographic parameters is needed to ensure a good resolution of analytes. In addition to mass spectrometry, ultraviolet/visible absorbance is a commonly used detection techniques with HPLC. Most microcystin congeners have similar absorption profiles between 200 and 300 nm. The wavelength of the UV/visible detector can be set at these values to record the responses of microcystins in sample extracts separated by the HPLC. The retention time, UV spectra and peak area of commercially available or laboratory standards is the basis of identification and quantification of microcystins using HPLC-UV/visible detection. However, due to the limited number of commercially available standards, the toxins are often quantified by comparison to an microcystin-LR standard and reported in terms of microcystin-LR equivalence. HPLC-UV/visible is susceptible to interferences from natural organic materials (NOMs). Detection limits will depend partially on the sample volume extracted, the concentration of the toxins, and the presence of interfering contaminants.

A variety of antibodies have been isolated against microcystin-LR and microcystin-RR, as well as recombinant antibody fragments and antibodies against the amino acid ADDA. Commercial ELISA kits that contain all of the reagents needed for analysis have also been developed and typically provide a cross reactivity chart for some of the congeners (i.e., microcystin-LR, -RR, YR, nodularin) that are commonly found in water. These range from 50-85% for microcystin-RR, 35-181% for microcystin-YR and 10-124% for microcystin-LA. Detection of the total microcystins will be expressed as the sum of the congeners provided from ADDA ELISA. The methods detection limit (MDLs) of several commercial laboratory ELISA kits have been reported to range from 0.04 to 0.2 μ g/L for microcystin-LR. Commercial ELISA kits generally have quantitation ranges from 0.2 (LOQ) to an upper limit of 5 μ g/L. Two high sensitivity ELISA plate kits have become commercially available with MDLs ranging from 0.04 to 0.05 μ g/L.

PPIAs are used with a variety of detection methods and substrates including radioactive detection assays using ^{32}P -radiolabelled substrates and colorimetric assays using p-nitrophenol phosphate as the substrate. The method has also been adopted for fluorescence measurements using the substrates methylumbelliferyl phosphate. The detection limit of total microcystins, reported as microcystin-LR equivalents (microcystin-LR_{equiv}) using radiometric protein phosphatase assays is approximately 0.1 $\mu\text{g/L}$ or less, and using colorimetric PP1 inhibition assays range between 10 to 20 ng/mL (0.01 to 0.02 $\mu\text{g/L}$).

Rapid tests for the identification of the presence of microcystins in water have been developed for use in the field. Field test kits can be used as a presence/absence tool for determining if a bloom is toxic or if treatment plant operations need to be adjusted during a bloom event but do not currently have sufficient sensitivity at microcystin concentrations below 1 $\mu\text{g/L}$ to be used for treated water analyses. Commercially-available test kits use a variety of methods including immunochromatography (test strips), ELISA, and phosphatase inhibition to estimate the level of microcystins in a water sample. In general, the results of field test kits should be considered qualitative and should only be used to conduct a preliminary assessment of microcystin levels. The applicability of test kits is between 1 and 5 $\mu\text{g/L}$ of microcystins with a detection limit of approximately 0.5 $\mu\text{g/L}$. Several field test kits do not include a lysing agent and, therefore, only determine the presence of extracellular microcystins. When using these field test kits, users should consult the manufacturer regarding an appropriate lysing technique if the detection of both intracellular and extracellular microcystins is required.

A new approach using laser diode thermal desorption-atmospheric pressure chemical ionization interface coupled to tandem mass spectrometry (LDTD-APCI-MS/MS) has been developed for the analysis of total microcystins in complex environmental matrices. The method is based on oxidation of the MCs in a sample using potassium permanganate under alkaline conditions to produce 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB). MMPB is then extracted and directly injected (no chromatographic separation) into the LDTD-APCI-MS/MS system. This approach results in ultra-fast sample analysis with simple sample preparation, reducing time and material costs associated with chromatographic separation. This method does not require individual MC standards, but similar to ELISA and PPIA, the results do not provide information on the identity of the individual MC congeners. The MDL and LOQ are 0.2 and 0.9 $\mu\text{g/L}$, respectively (Roy-Lachapelle et al., 2014).

6.0 TREATMENT TECHNOLOGIES

The information below is adapted from the Health Canada Guidelines for Cyanobacteria Toxins in Drinking Water, available later in 2015.

Detailed information on the operational considerations of a variety of treatment methods can be found in the EPA *Drinking Water Treatability Database for Microcystins* (U.S. EPA, 2007); the *International Guidance Manual for the Management of Toxic Cyanobacteria* (GWRC, 2009) available at <http://www.waterra.com.au/cyanobacteria-manual/PDF/GWRCGuidanceManualLevel1.pdf>, and *Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities* (Newcombe et al., 2010) available at [http://www.researchgate.net/profile/Lionel_Ho/publication/242740698_Management_Strategies_for_Cyanobacteria_\(Blue-Green_Algae\)_A_Guide_for_Water_Utilities/links/02e7e52d62273e8f70000000.pdf](http://www.researchgate.net/profile/Lionel_Ho/publication/242740698_Management_Strategies_for_Cyanobacteria_(Blue-Green_Algae)_A_Guide_for_Water_Utilities/links/02e7e52d62273e8f70000000.pdf)

For additional information on treatment strategies commonly used or being considered by water systems vulnerable to cyanotoxins, please see *Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water* (U.S. EPA, 2015b).

6.1 Management and Mitigation of Cyanobacterial Blooms in Source Water

Algaecides can be applied to lakes and reservoirs to mitigate algal blooms, including cyanobacteria. In most cases, depending on the cyanobacteria species present, the application of algaecides has the potential to compromise cell integrity releasing cyanotoxins into the source waters. Chemical treatment to control blooms in drinking water sources in the early stages of the bloom when cyanobacterial concentrations are still relatively low (usually under 5,000 to 15,000 cells/mL) (WHO, 1999), are less likely to release significant cyanotoxin concentrations upon cell lysis and is able to mitigate or prevent a cyanobacterial bloom from proliferating as the season progresses. If harmful cyanobacterial blooms occur, utilities may take action to investigate alternative source water sources, change intake locations or levels to withdraw source water with minimal cyanotoxin concentrations, or investigate methods of destratification in the water source. Purchasing water from a neighboring interconnected water system that is unaffected by the bloom may also be an option for some systems.

Clays and commercial products such as aluminum sulfate (alum) have been used for the management of blooms in source waters. Alum treatment efficiency depends on the alum dose and the type of flocculant. Aeration and destratification have also been used to treat cyanobacterial blooms, usually in smaller water bodies (from one acre to several tens of acres). Active mixing devices, diffuse air bubblers, and other means of reducing stratification have proven to be effective in controlling outbreaks and persistence of blooms in relatively small shallow impoundments (around <20 feet deep). These strategies can be applied to the entire source water body or to just a portion of the lake depending on the need, and size and depth of the water body relative to the source water intake(s).

Hydrogen peroxide (H₂O₂) has been used as an algaecide in source water because of a rapid reaction time (90% of bloom collapsed in 3 days and 99% in 10 days), and environmentally safe reaction products (oxygen and water) (Wang et al., 2012; Matthjis et al; 2012). The

drawbacks (aside from cell lysis) are that oxidant breakdown is so rapid that repeated applications are needed. Further understanding of this technique is needed (Matthjis et al., 2012).

The use of ultrasonic sound waves to disrupt cyanobacterial cells has also been investigated as a potential source water treatment option (Rajasekhar et al., 2012). It is environmentally friendly compared to chemical treatment strategies. The technique has also been reported to be capable of degrading microcystin-LR (Song et al., 2005). Drawbacks include that application frequencies are difficult to calculate and are system-specific; and that applications on large scale require more powerful and, therefore, more expensive equipment. Sonication shows potential for use in cyanobacterial bloom management, but further study to determine effective operating procedures is needed before it can be considered as a feasible approach (Rajasekhar et al., 2012).

Excess nutrients are thought to be a primary driver of cyanobacterial blooms. Long-term prevention of cyanobacterial blooms likely requires reductions in nutrient pollution. Excess nitrogen and phosphorus in aquatic systems can stimulate blooms and create conditions under which harmful cyanobacteria thrive. Thus, managing nutrient pollution sources within a watershed in addition to waterbody-specific physical controls (in systems that are amenable to those controls) tends to be the most effective strategy. Nutrient pollution can be from urban, agricultural, and atmospheric sources and, therefore, reductions can be achieved through a variety of source control technologies and best management practices.

6.2 Drinking Water Treatment

Effective treatment of cyanotoxins in drinking water includes the evaluation and selection of appropriate treatment methods. Any variation in treatment methods aimed at reducing toxins concentrations need to be tailored to the type(s) of cyanobacteria present and the site-specific water quality (e.g. pH, temperature, turbidity, presence of natural organic material (NOM), etc.), the treatment processes already in place, and the utility's multiple treatment goals (e.g., turbidity and total organic carbon (TOC) removal, disinfection requirements, control of disinfection by-products (DBP) formation, etc.). Utilities need to have an understanding of the type and concentration of cyanotoxins present in the source water and should conduct site-specific evaluations such as jar testings and piloting in order to determine the most effective treatment strategy. Potential target parameters include: chlorophyll-a, turbidity, cyanobacterial cells and extracellular and intracellular toxins. Care should be taken to avoid cell lysis. To remove both intracellular and extracellular toxins from drinking water, a multi-barrier approach is required, which may consist of conventional filtration for intracellular cylindrospermopsin removal and additional processes such as activated carbon, biodegradation, advanced oxidation, and small-pore membrane processes (e.g. nanofiltration and reverse osmosis), for the removal or oxidation of extracellular cylindrospermopsin. The most effective way to deal with cyanobacteria cells and their toxins, is to remove the cells intact, without damaging them, to prevent the release of additional extracellular toxins into the water.

6.2.1 Conventional Treatment for Microcystins

In the absence of cell damage, conventional treatment employing coagulation, flocculation, clarification (sedimentation or dissolved air flotation) and rapid granular filtration can be effective at removing intact cells and the majority of intracellular toxins (cell bound) (Chow et al., 1998; Newcombe et al., 2015). However, if toxins are released into solution, a combination of conventional treatment processes with oxidation, adsorption, and/or advanced treatment needs to be considered to treat both intracellular and extracellular cyanotoxins.

The efficiency of the conventional treatment processes to remove cyanobacterial cells and intracellular microcystins has been shown to vary from 60 to 99.9%. Factors that impact removal include the cyanobacterial species and cell density, coagulant type and dose, pH, NOM, and operational parameters such as flocculation time, frequency of filter backwashing and clarifier sludge removal (Vlaski et al., 1996; Hoeger et al., 2004; Jurczak et al., 2005; Zamyadi et al., 2012a, 2013c; Newcombe et al., 2015). Typically, 60 to 95% of cells and intracellular microcystins can be removed during sedimentation with as much as 99.9% removal achieved through filtration (Lepisto et al., 1994; Drikas et al., 2001; Hoeger et al., 2004; Newcombe et al., 2015). The efficiency of coagulation and clarification for cell removal is dependent on pH, coagulant type and dose and the morphological characteristics of the cyanobacteria. Rapid sand filtration without pre-treatment (i.e., direct filtration, without coagulation/clarification) is not effective for cyanobacterial cell removal.

If operated properly, conventional treatment (coagulation, flocculation, clarification and filtration), does not cause cell lysis or increases in the extracellular microcystin concentrations of treated water (Chow et al., 1998, 1999; Drikas, 2001; Sun et al., 2012). Drinking water treatment plants utilizing conventional treatment followed by oxidation or activated carbon may remove both intracellular and extracellular microcystins up to 99.99% of total microcystins to achieve concentrations below 0.1 µg/L in treated water (Karner et al., 2001; Lahti et al., 2001; Hoeger et al., 2005; Jurczak et al., 2005; Rapala et al., 2006; Zamyadi et al., 2013a). Conventional treatment is generally considered to have limited effectiveness for the removal of the extracellular microcystins. Therefore, additional processes such as adsorption, chemical oxidation, biodegradation or reverse osmosis, and nanofiltration are required to remove extracellular microcystins.

Although microfiltration and ultrafiltration membranes can remove both cyanobacterial cells and intracellular microcystins, removal of extracellular microcystins using ultrafiltration is variable (35 to 70%) and microfiltration is not effective (Gijssbertsen-Abrahamse et al., 2006; Dixon et al., 2011a, b). Nanofiltration and reverse osmosis membranes can achieve high removals of intracellular and extracellular microcystins, from 82% to complete removal (Westrick et al., 2010; Dixon et al., 2010). Pore size, among others, is an important factor in removal efficiency for these processes.

Successful removal of cyanobacterial cells and intracellular microcystins will depend on proper operations of the conventional treatment processes (Hoeger et al., 2004; Dugan and Williams, 2006; Ho et al., 2013; Zamyadi et al., 2012a, 2013c). Operational considerations for removing cyanobacterial cells using coagulation, flocculation and clarification are similar to

considerations for achieving effective particle removal. The appropriate coagulant and coagulation pH should be determined through jar-testing to maximize cell removal. In jar-testing, the NOM, chlorophyll-a, or cyanobacterial cell count can be used to optimize the coagulation conditions for cyanobacterial cell removal (Sklenar et al., 2014; Newcombe et al., 2015). Sufficient mixing should be provided at the point of chemical addition to ensure rapid and uniform contact, and an appropriate mixing speed should be determined to optimize the flocculation process (GWRC, 2009). It is important to minimize the potential for the accumulation of cyanobacterial cells as scums at the surface of sedimentation basins and filters (Zamyadi et al., 2012a, 2013c).

Effective sludge removal from sedimentation/clarification processes is important to minimize the release of intracellular and extracellular microcystins into the surrounding waters, as significant cell numbers can accumulate within the sludge, and cells contained within the sludge can lyse rapidly (Drikas et al., 2001; Ho et al., 2013; Zamyadi et al. 2012a). It has been reported that accumulation of cyanobacterial cells and microcystins in clarifiers can lead to their breakthrough into filter effluent. In addition, cell lysis can occur in the clarifier sludge, increasing the extracellular concentration of microcystins in the treatment plant. Therefore sludge management (decreased sludge age) in clarifiers and increased frequency of backwashing of filters is important because settled/filtered cells can remain viable and possibly multiply over a period of at least 2 to 3 weeks. Within 1 day, some cells in the sludge can lyse and release NOM and taste and odor compounds, in addition to cyanotoxins (Newcombe et al., 2015). Additionally, backwash water from the filters may contain cyanobacterial cells and/or extracellular microcystins; hence, care needs to be taken if spent backwash water is recirculated to the beginning of the treatment process to prevent the reintroduction of cells and toxins into the treatment train. Although longer filter run-times are typically desirable between backwashing, during periods of high algal concentrations, cells can accumulate in the filter, which can potentially lead to a significant amount of extracellular microcystins released into the filtered water. The optimum balance between maximizing water production and minimizing the risk of toxin breakthrough will be plant-specific.

6.2.2 Adsorption

Adsorption processes, such as granular activated carbon (GAC) or powdered activated carbon (PAC), are effective at removing extracellular microcystins but are not capable of removing intact cells and intracellular toxins (Lambert et al., 1996; Newcombe, 2002; Newcombe et al., 2003). Removal through adsorption depends on many factors including the type of activated carbon used, the microcystin congener and water quality conditions. In general, mesoporous carbons (such as chemically-activated wood-based carbons) are the most effective for the removal of microcystins (Newcombe et al., 2010). Other factors such as the type of microcystin congener present, the raw water quality (i.e., NOM and pH) and contact time affect microcystins removal efficiency when using activated carbon processes. In addition, shortened filter run times or filter overload may happen during cyanobacteria blooms. Therefore, water treatment plants should conduct jar-testing to determine the most effective activated carbon dose, type, and feed point prior to the application without affecting other water quality parameters and treatment processes (Sklenar et al., 2014).

The performance of GAC filtration for extracellular microcystin removal depends upon the empty bed contact time (EBCT), carbon age, carbon pore size, and raw water quality characteristics such as NOM and pH, as well as the microcystin variant (Newcombe, 2002; Newcombe et al., 2003; Ho and Newcombe, 2007; Wang et al., 2007). Solution chemistry can also affect microcystin-LR adsorption onto GAC. Enhanced removal of microcystin-LR has been observed at lower pH (2.5 versus 6.5) due to either precipitation or reduced solvency effect (Pendleton et al., 2001).

Removal of extracellular microcystins by PAC can be highly effective (up to 95%) depending on the microcystin congener and concentration, the PAC type and dose, the contact time and the water quality characteristics such as TOC (Newcombe et al., 2003; Cook and Newcombe; 2008; Ho et al., 2011). According to Newcombe et al. (2010), a PAC dose of 20 mg/L and a contact time of at least 45 minutes should be considered for removal of most extracellular microcystins (with the exception of microcystin-LA).

6.2.3 Chemical Oxidation

Chemical oxidation using chlorine, potassium permanganate, or ozonation can be effective at oxidizing extracellular microcystins, but can also impair cell integrity, resulting in an increase in concentrations of extracellular microcystins in drinking water. By applying conventional filtration (or other filtration process) first to remove the majority of intact cells, the extracellular microcystin concentration is less likely to increase due to cell lysis when water is treated with oxidants. In cases where pre-oxidation (oxidant applied anywhere along the treatment process prior the filter influent) is practiced, it may need to be discontinued during an algal bloom or adjustments to the oxidant type and doses may be needed to minimize cell rupture prior to filtration (Newcombe et al., 2015).

The effectiveness of chemical oxidation of microcystins depends on the type of oxidant, dose, contact time, microcystin congener and water quality characteristics such as pH and dissolved organic carbon (DOC) (GWRC, 2009; Sharma et al., 2012). Laboratory-scale experiments have demonstrated that the general trend for the effectiveness of cyanobacterial cell and extracellular microcystin oxidation to be: ozone>permanganate>chlorine>chlorine-based oxidants (Acero et al., 2005; Rodriguez et al., 2007a, b; Ding et al., 2010; Sharma et al., 2012;). However, selection of the most appropriate oxidant for microcystins should be based on the characteristics of each water source, the disinfection requirements, and potential formation of disinfection by-products (DBPs) (Sharma et al., 2012).

It is also important to recognize that the use of oxidants may result in the formation of DBPs and should be considered when selecting a strategy for oxidizing microcystins (Merel et al., 2010; Zamyadi et al., 2012b; Wert et al., 2013). For example, ozone and chlorine dioxide can result in the formation of inorganic DBPs, such as bromate and chlorite/chlorate, respectively. Additionally, modifying pre-oxidation practices may compromise other treatment objectives (e.g., turbidity removal), and should be considered.

The oxidation of microcystins by chlorine has been found to be highly effective (>90% removal) under experimental conditions (Ho et al., 2006a; Acero et al., 2008; Merel et al., 2009; Sorlini and Collivignarelli, 2011). However, the effectiveness of chlorination on the oxidation of microcystins depends upon the chlorine dose, contact time, pH, temperature, and other water quality characteristics (Sharma et al., 2012). Several studies have found that microcystins are efficiently oxidized if pH is maintained below 8, the chlorine dose is greater than 3 mg/L and 0.5 to 1.5 mg/L of free chlorine residual is present after 30 minutes of contact time (Nicholson et al., 1994; Acero et al., 2005; Ho et al., 2006a; Xagorarakis et al., 2006; Newcombe et al., 2010). However, much higher chlorine doses (2 to 10 mg/L) are required to lyse the cyanobacterial cell and then oxidize the previously cell-bound microcystins (Zamyadi et al., 2013b).

The oxidation of microcystins in water by permanganate is one of the more effective processes for oxidizing extracellular microcystins in water (Sharma et al., 2012). Rodriguez et al. (2007a) exhibited a 90% oxidation of microcystin-LR at a dose of 1.0 mg/L, a contact time of 60 minutes, a pH of 8, and a temperature of 20°C. Complete oxidation occurred at a dose of 1.5 mg/L (Rodriguez et al., 2007a). Treatment plants considering potassium permanganate for oxidation of microcystins should be aware that permanganate can discolor water when it is present in excess of 0.05 mg/L. Therefore, dosage control and process monitoring (e.g., visual inspection of the basin effluent color, measuring permanganate residual) is important in avoiding consumer complaints (MWH, 2012).

The oxidation of microcystins in water by ozone has been shown to be highly effective (greater than 90% oxidation) in laboratory-scale studies (Rositano et al., 2001; Shawwa and Smith, 2001; Brooke et al., 2006). The efficacy depends on temperature, pH, ozone dose, contact time, and other water quality characteristics such as DOC and alkalinity (Sharma et al., 2012). Utilities should also be aware that the use of ozone may result in the formation of bromate and other DBPs.

Monochloramine is a weaker oxidant than chlorine and is not an effective treatment barrier for microcystins (Westrick et al., 2010).

Most laboratory studies have found that chlorine dioxide (ClO₂) is not effective for oxidizing extracellular microcystins (Kull et al., 2004, 2006; Ding et al., 2010; Sorlini and Collivignarelli, 2011) or cyanobacterial cells and intracellular microcystins (Ding et al., 2010; Wert et al., 2014) at dosages (1-2 mg/L) and contact times typically applied to drinking water.

6.2.4 Other Filtration Technologies

Biological filtration, using either biologically-active sand or activated carbon, has been shown to be effective for the removal of extracellular microcystins in bench- and pilot-scale studies (Keijola et al., 1998; Bourne et al., 2006; Ho et al., 2006b, 2008, 2012) and in limited full-scale studies (Grutzmacher et al., 2002, Rapala et al., 2006). The removal of intact cyanobacterial cells and their associated intracellular toxins through physical straining in slow sand filters has also been documented (Grutzmacher et al., 2002; Pereira et al., 2012). Biological filters also have the capability to remove particulate including intact cyanobacterial cells. Bank filtration may also be effective for the removal of microcystins (Lahti et al., 1998; Schijven et al., 2002). A detailed

review of biological treatment options for cyanotoxin removal conducted by Ho et al., (2012b) identified the type and concentration of microcystin-degrading bacteria, concentration of microcystins, and temperature as key factors that influence the efficiency of biological filtration for the removal of microcystins. In addition, the concentration of other organic matter within the source water may inhibit biodegradation, as microcystins may be a secondary substrate in the presence of NOM. Particle size, chemical composition and roughness or topography of the surface of the media used for filtration have also been identified as important factors for biofilm growth and ultimately the biodegradation of microcystins (Wang et al., 2007, Ho et al., 2012).

Membrane filtration including microfiltration (MF) and ultrafiltration (UF) can achieve greater than 98% removal of cyanobacterial cells and intracellular microcystins (Chow et al., 1997; Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010; Sorlini et al., 2013). Nanofiltration (NF), reverse osmosis (RO) and, to a lesser extent UF, can be used for both intracellular and extracellular microcystin removal (Neumann and Weckesser, 1998; Lee and Walker, 2008; Dixon et al., 2011a,b). The performance of membrane filtration for microcystin removal depends on characteristics of the membrane such as molecular weight cut-off (MWCO) and hydrophobicity, initial concentration, size and molecular weight of the microcystins, and operating parameters such as flux, recoveries and degree of fouling. It is recommended that cyanobacterial cells are removed prior to reverse osmosis to prevent membrane clogging and fouling.

Laboratory and pilot-scale studies have demonstrated that MF and UF can remove greater than 98% of cyanobacterial cells (Chow et al., 1997, Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010; Sorlini et al., 2013), and ultrafiltration can be moderately effective (35-70%) for removal of extracellular microcystins (Lee and Walker, 2008). Several studies have also demonstrated that the release of intracellular microcystins from the shear stress on cyanobacterial cells during MF and UF is possible, although it generally results in permeate microcystin concentration increases of less than 12 percent (Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010).

The removal of extracellular microcystins by NF and RO is very effective (greater than 90%) and depends on the MWCO, as the filtration of microcystins occurs via size exclusion (Gijsbertsen-Abrahamse et al., 2006).

6.2.5 Combined Treatment Technologies

In practice, full-scale treatment plants use a combination of treatment technologies (i.e., conventional filtration and chemical oxidation) in order to remove both intracellular and extracellular microcystins. Data indicate that utilities can effectively remove both intracellular and extracellular microcystins to achieve concentrations below 0.1 µg/L (Lahti et al. 2001; Boyd and Clevenger, 2002; Zurawell, 2002; Hoeger et al., 2005, Jurczak et al., 2005; Rapala et al., 2006; Haddix et al., 2007; Nasri et al., 2007; Zamyadi et al., 2013c). However, some studies have shown that the presence of high concentrations of cells (i.e., 10⁵ cells/mL) and/or microcystins in raw water (100 µg/L) may be challenging for treatment plants to reduce concentrations to below 0.1 µg/L (Tarczyrska et al., 2001; Zamyadi et al., 2012a).

In most cases, utilities will be able to effectively remove intracellular microcystins with processes that are already in place (e.g., conventional treatment) when they are operated with a focus on cyanobacteria cell or NOM removal. Extracellular microcystins may also be removed in many treatment plants by using existing treatment such as chlorination after filtration or by the addition of PAC following conventional treatment (Carriere et al., 2010). Although it is possible to remove both intracellular and extracellular microcystins effectively using a combination of treatment processes, the removal efficiency can vary considerably. Utilities need to ensure that they are utilizing their existing treatment processes to their fullest capacity for removal of both cyanobacterial cells and extracellular microcystins and that appropriate monitoring is being conducted to ensure that adequate removal is occurring at each step in the treatment process.

6.3 Point-of-Use (POU) Drinking Water Treatment Units

Limited information is available on residential treatment units for the removal of cyanobacteria cells and microcystins. A study using common water filtration and purification systems found that the efficacy of POU filtration devices to remove microcystin (LR) varies considerably with the type of device being used (Pawlowicz et al., 2006). Microcystin-LR was successfully removed using carbon filters allowing only 0.05 to 0.3% of the toxin load to pass through the filter. However, more than 90% of microcystin-LR passed through string-wound filters and pleated paper. According to the authors, the use of carbon home filter devices tested in this study may provide additional protection beyond that provided by the drinking water treatment plant against human exposure to microcystin-LR. Additional studies are recommended to assess the efficacy of POU drinking water treatment units for other cyanotoxins and under other conditions. Third-party organizations are currently developing certification standards to test POU devices to evaluate how well they remove cyanotoxins from drinking water treatment units. Those standards are expected in the near future.

More information about treatment units and the contaminants they can remove can be found at <http://www.nsf.org/Certified/DWTU/>.

7.0 REFERENCES

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