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Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin

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Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

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ABBREVIATIONS AND ACRONYMS

BMD	Benchmark Dose
BMDL	Benchmark Dose Level
BW	Body Weight
CAS	Chemical Abstracts Service
CCL	Contaminant Candidate List
CWA	Clean Water Act
CYP450	Cytochrome P450
DAF	Dissolved Air Flotation
DBP	Disinfection By-Products
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
DWI	Drinking Water Intake
ELISA	Enzyme Linked Immunosorbent Assay
EPA	U.S. Environmental Protection Agency
g	Gram
GAC	Granular Activated Carbon
GFR	Glomerular Filtration Rate
HA	Health Advisory
HAB	Harmful Algal Bloom
HESD	Health Effects Support Document
HPLC	High Performance Liquid Chromatography
ICR	Institute for Cancer Research
i.p.	Intraperitoneal
kg	Kilogram
K_{oc}	Organic Carbon:Water Partition Coefficient
K_{ow}	Octanol:Water Partition Coefficient
L	Liter
LC	Liquid Chromatography
LCAT	Lecithin Cholesterol Acyl Transferase
LC-ESI/MS	Liquid Chromatography Tandem Electrospray Ionization Mass Spectrometry
LCMRL	Lowest Concentration Method Reporting Limit
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LOAEL	Lowest-Observed-Adverse-Effect Level
MCH	Mean Corpuscular Hemoglobin
µg	Microgram
µm	Micromole
MNBNC	Micronucleated Binucleated Cells
mg	Milligram
mL	Milliliter
mmol	Millimole
MOA	Mode of Action
MWCO	Molecular Weight Cut-off
N	Nitrogen

N/A	Not Applicable
NARS	National Aquatic Resource Surveys
ng	Nanogram
NHANES	National Health and Nutrition Examination Survey
NLA	National Lake Assessment
NOAEL	No-Observed-Adverse-Effect Level
NOM	Natural Organic Material
OECD	Organization for Economic Cooperation and Development
P	Phosphorus
PAC	Powdered Activated Carbon
PDA	Photodiode Array
pg	Picogram
POU	Point-of-Use
RBC	Red Blood Cell
RfD	Reference Dose
SDWA	Safe Drinking Water Act
SHE	Syrian Hamster Embryo
SPE	Solid Phase Extraction
TOC	Total Organic Carbon
TOXLINE	Toxicology Literature Online
UF	Uncertainty Factor
USACE	U.S. Army Corps of Engineers
USGS	U.S. Geological Survey
UV	Ultraviolet

EXECUTIVE SUMMARY

Cylindrospermopsin is a toxin produced by a variety of cyanobacteria including: *Cylindrospermopsis raciborskii (C. raciborskii), Aphanizomenon flos-aquae, Aphanizomenon gracile, Aphanizomenon ovalisporum, Umezakia natans, Anabaena bergii, Anabaena lapponica, Anabaena planctonica, Lyngbya wollei, Rhaphidiopsis curvata,* and *Rhaphidiopsis mediterranea*.

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 cylindrospermopsin blooms, both in fresh and marine water systems. These species do not tend to form visible surface scums and the highest concentrations of cells occurs below the water surface. Cylindrospermopsin may be retained within the cell, but most of the time it is found in the water (extracellular) or attached to particulates present in the water.

This Health Advisory (HA) for the cyanobacterial toxin cylindrospermopsin 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 animal studies demonstrate absorption of cylindrospermopsin from the intestinal tract primarily in the liver, but also in the kidney and spleen. Limited data are available on the metabolism of cylindrospermopsin, but evidence indicates that metabolism and toxicity are mediated by the hepatic cytochrome P450 (CYP450) enzyme system. The periacinar region of the liver, an area where substantial CYP450-mediated xenobiotic metabolism occurs, appears to be the main target of cylindrospermopsin toxicity and where cylindrospermopsin and its metabolites bind to proteins. The few studies evaluating elimination suggest that cylindrospermopsin is rapidly eliminated primarily in the urine, but also in feces.

The main source of information on the toxicity of cylindrospermopsin in humans is from qualitative reports of a hepatoenteritis-like illness attributed to acute or short-term consumption of drinking water containing *C. raciborskii*. Symptoms reported include fever, headache, vomiting, bloody diarrhea, hepatomegaly, and kidney damage with loss of water, electrolytes and protein. No reliable data are available on the exposure levels of cylindrospermopsin that induced these effects.

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

The U.S. Environmental Protection Agency (EPA) identified a study by Humpage and Falconer (2002, 2003) conducted on mice as the critical study used in the derivation of the reference dose (RfD) for cylindrospermopsin. The critical effects identified in the study areincreased kidney weight and decreased urinary protein. The NOAEL (No Observed Adverse

Effect Level) was determined to be 30 µg/kg/day based on kidney toxicity. The total uncertainty factor (UF) applied to the NOAEL was 300. This was based on a UF of 10 for intraspecies variability, a UF of 10 for interspecies variability, and a UF of 3 ($10^{½}$) to account for deficiencies in the database.

EPA is issuing a Ten-day HA for cylindrospermopsin based on the Humpage and Falconer (2002, 2003) 11-week study. Studies of a duration of 7 days up to 30 days are typically used to derive Ten-day HAs. In this case, a subchronic study was determined to be suitable for the derivation of the HA. Although the duration of the Humpage and Falconer (2002, 2003) study is longer (77 days) than the studies typically used for the derivation of a Ten-day HA, the short-term studies available for cylindrospermopsin (Shaw et al., 2001; Reisner et al., 2004) are not suitable for quantification; however, effects observed in these studies are the same or similar to the Humpage and Falconer study (2002, 2003) and occur at similar doses.

The short-term HA is consistent with the available data and most appropriately matches human exposure scenarios for cyanobacterial blooms in drinking water. Cyanobacterial blooms are usually seasonal, typically occurring from May through October. In the presence of algal cell pigments, photochemical degradation of cylindrospermopsin can occur rapidly, with reported half-lives of 1.5 to 3 hours. In the absence of pigments, however, there is little degradation. The biodegradation of cylindrospermopsin in natural water bodies is a complex process that can be influenced by many environmental factors, including concentration, water temperature and the presence of bacteria. Half-lives of 11 to 15 days and up to 8 weeks have been reported for cylindrospermopsin in surface waters. 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 0.7 µg/L and for school-age children through adults is 3 µg/L for cylindrospermopsin. The two advisory values use the same toxicity data (RfD) and represent differences in drinking water intake and body weight for different human 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 (U.S. EPA's Exposure Factors Handbook, 2011a). The second advisory value is based on the mean body weight and the 90th percentile drinking water consumption rate 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 adult population to the health effects of cylindrospermopsin. 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.

No epidemiological studies of the association of cylindrospermopsin and cancer are available. Also, no chronic cancer bioassays of purified cylindrospermopsin in animals were identified. Therefore, under the U.S. EPA's (2005) Guidelines for Carcinogen Risk Assessment, there is *inadequate information to assess carcinogenic potential* of cylindrospermopsin.

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 Cylindrospermopsin* (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 microcystins to assist State and local officials in evaluating risks from these contaminants in drinking water.

Internationally, three countries and two U.S. states have developed drinking water guidelines for cylindrospermopsin, as shown in Table 1-1 and Table 1-2, respectively.

Table 1-1. International Guideline Values for Cylindrospermopsin

Country	Guideline Value	Source
Australia	1 µg/L	Australian Drinking Water Guidelines 6 (NHMRC, NRMMC, 2011)
New Zealand	1 µg/L	Drinking-water Standards for New Zealand 2005 (Ministry of Health, 2008)
Brazil	15 µg/L (recommended)	Guidelines for Drinking Water Quality, Official LA Report's, Regulation MS N 518/2004 (Brasil, 2009)

Table 1-2. State Guideline Values for Cylindrospermopsin

State	Guideline Value	Source
Ohio	1 µg/L	State of Ohio Public Water System Harmful Algal Bloom Response Strategy (OHEPA, 2014)
Oregon	1 µg/L	Public Health Advisory Guidelines, Harmful Algae Blooms in Freshwater Bodies. (OHA, 2015)

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 Cylindrospermopsin* (U.S. EPA, 2015a).

2.1 Cyanobacteria and Production of Cylindrospermopsin

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, pteriodophytes, 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 (nitrogen, phosphorus 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).

Cylindrospermopsin is a toxin produced by a variety of cyanobacteria including: *Cylindrospermopsis raciborskii (C. raciborskii), Aphanizomenon flos-aquae, Aphanizomenon gracile, Aphanizomenon ovalisporum, Umezakia natans, Anabaena bergii, Anabaena lapponica, Anabaena planctonica, Lyngbya wollei, Rhaphidiopsis curvata,* and *Rhaphidiopsis mediterranea.*

2.2 Physical and Chemical Properties

The cyanotoxin cylindrospermopsin is a tricyclic alkaloid with the following molecular formula $C_{15}H_{21}N_5O_7S$ (Ohtani et al., 1992) and a molecular weight of 415.43 g/mole. It is zwitterionic (i.e., a dipolar ion with localized positive and negative charges) (Ohtani et al., 1992) and is believed to be derived from a polyketide that uses an amino acid starter unit such as glycocyamine or 4-guanidino-3-oxybutyric acid (Duy et al., 2000). The chemical structure of cylindrospermopsin is presented in Figure 2-1. Two naturally occurring congeners of cylindrospermopsin have been identified (Figure 2-2): 7-epicylindro-spermopsin (the epimer of cylindrospremopsin) and 7-deoxycylindrospermopsin (Norris et al., 1999; de la Cruz et al., 2013). Recently, Wimmer et al. (2014) identified two new analogs, 7-deoxy-desulfo-

Figure 2-1. Structure of Cylindrospermopsin (de la Cruz et al., 2013)

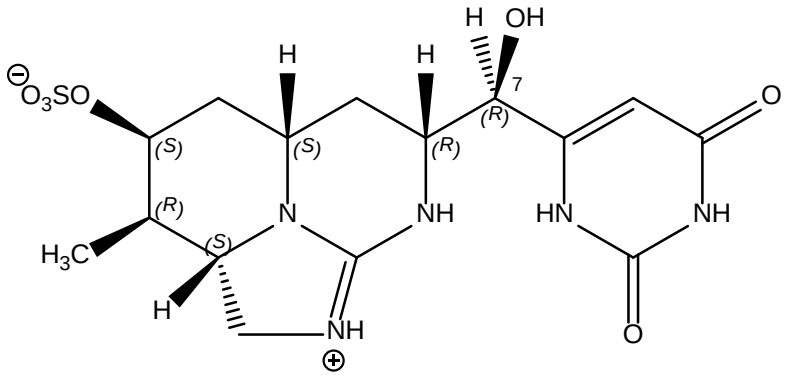
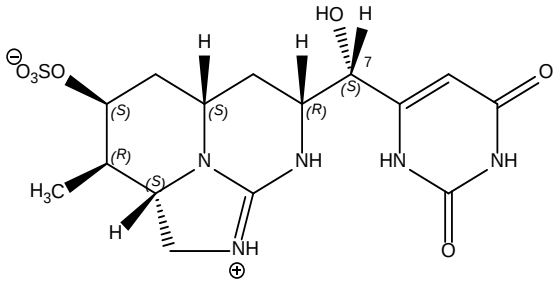
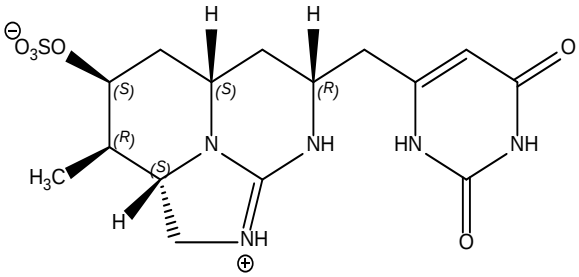


Figure 2-2. Structurally Related Cylindrospermopsins (de la Cruz et al., 2013)

7-epi-cylindrospermopsin



7-deoxycylindrospermopsin



cylindrospermopsin and 7-deoxy-desulfo-12-acetylcylindrospermopsin, from the Thai strain of *C. raciborskii*. However, it is not clear if these are cylindrospermopsin congeners, precursors or degradation products. Chlorination of water containing cylindrospermopsin can produce 5-chlorocylindrospermopsin and cylindrospermic acid.

The physical and chemical properties of cylindrospermopsin are presented in Table 2-1. Cylindrospermopsin generally exists in a zwitterionic state (with both positive and negative ions) and is highly soluble in water (Moore et al., 1998, Chiswell et al., 1999). Cylindrospermopsin is isolated for commercial use mostly from *C. raciborskii.* Other physicochemical properties of cylindrospermopsin in the environment such as vapor pressure, boiling and melting points, soil organic carbon-water partition coefficient (Koc), octanol-water partition coefficient (Kow), and vapor pressure and Henry's Law constant are unknown. Available information on the chemical

Table 2-1. Chemical and Physical Properties of Cylindrospermopsin

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

Sources: Chemical Book, 2012; TOXLINE, 2012

breakdown, biodegradation and mobility of cylindrospermopsin in the environment is discussed in the Environmental Fate section.

2.3 Sources and Occurrence

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 cylindrospermopsin blooms (Paerl and Huisman, 2008; Paerl and Otten, 2013). Although cylindrospermopsin-producing cyanobacteria (such as *C. raciborskii)* occur mostly in tropical or subtropical regions, they have also been found in warmer temperate regions, both in fresh and marine water systems. These species do not tend to form visible surface scums and the highest concentrations of cells occurs below the water surface (Falconer 2005). Cylindrospermopsin may be retained within the cell, but most of the time it is found in the water (extracellular) or attached to particulates present in the water (Chiswell et al., 2001).

2.3.1 Occurrence in Surface Water

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 algal toxins, but did not include cylindrospermopsin. The United States Geological Survey (USGS) subsequently analyzed the stored samples collected during the NLA and reported that cylindrospermopsin was present in 5% of the samples; however, concentrations of cylindrospermopsin were not reported (Loftin and Graham, 2014). Future NARS plan to include other algal toxins, including cylindrospermopsin.

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

Many states monitor for harmful algal blooms (HABs). State monitoring efforts are expanding with greater awareness of the toxic effects of HABs. These monitoring efforts tend to focus on priority waters used for recreation or drinking water. Sampling is seasonal or on occasions when blooms are observed.

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

2.3.2 Occurrence in Drinking Water

The occurrence of cyanotoxins in finished drinking water depends on their levels in the raw source water and the effectiveness of the treatment methods used for removing cyanobacteria and cyanotoxins during the production of drinking water. Currently there is no federal or state program in place that requires monitoring for cyanotoxins at U.S. drinking water treatment plants. Therefore, data on the presence or absence of cyanotoxins in finished drinking water are limited.

EPA used information from the published literature to evaluate the potential occurrence of cylindrospermopsin in public water systems. In the single publication identified, the results of a 2000 survey of toxins in drinking water treatment plants in Florida were reported (Burns, 2008). In this survey, cylindrospermopsin was detected at concentrations ranging from 8 µg/L to 97 µg/L in nine finished drinking water samples.

2.4 Environmental Fate

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

2.4.1 Persistence

Cylindrospermopsin is relatively stable in the dark and at temperatures from 4°C to 50°C for up to five weeks (ILS, 2000). Cylindrospermopsin is also resistant to changes in pH and remains stable for up to eight weeks at pH 4, 7 and 10. In the absence of cell pigments, cylindrospermopsin tends to be relatively stable in sunlight, with a half-life of 11 to 15 days in surface waters (Funari and Testai, 2008). Cylindrospermopsin remains a potent toxin even after boiling for 15 minutes (Chiswell et al., 1999).

Degradation of cylindrospermopsin increases in the presence of cell pigments such as chlorophyll-a and phycocyanin. When exposed to both sunlight and cell pigments, cylindrospermopsin breaks down rapidly, more than 90% within 2 to 3 days (Chiswell et al., 1999). Cylindrospermopsin has been shown to be decomposed by bacteria in laboratory studies; the biodegradation is influenced by the toxin concentration, temperature and pH. Mohamed and Alamri (2012) reported that cylindrospermopsin was degraded by *Bacillus* bacteria and degradation occurred in 6 days at the highest toxin concentration (300 μg/L) and in 7 or 8 days at lower concentrations (10 and 100 μg/L, respectively). The biodegradation rate was also reported to depend on temperature and pH, with the highest rates occurring in warm waters (25 and 30°C) and neutral to slightly alkaline conditions (pH 7 and 8). Klitzke and Fastner (2012) confirmed the observations of Mohamed and Alamri (2012), noting that a decrease in temperature from 20 to 10°C slowed down degradation by a factor of 10. They also found that degradation slowed significantly under anaerobic conditions, with half-lives of 2.4 days under aerobic conditions and 23.6 days under anaerobic conditions.

2.4.2 Mobility

In sediments, cylindrospermopsin exhibits some adsorption to organic carbon, with little adsorption observed on sandy and silt sediments (Klitzke et al., 2011). The low adsorption of cylindrospermopsin reduces its residence time in sediments, thus reducing the opportunity for microbial degradation.

2.5 Nature of the Cylindrospermopsin Toxin

2.5.1 Toxicokinetics

Animal studies show that cylindrospermopsin is absorbed from the gastrointestinal tract (Humpage and Falconer, 2003; Shaw et al., 2000, 2001) and that the tissue distribution occurs

primarily to the liver, but also to the kidneys and spleen after intraperitoneal (i.p.) exposure (Norris et al., 2001).

The metabolism and toxicity of cylindrospermopsin is mediated by the hepatic cytochrome P450 (CYP450) enzyme system. The periacinar region of the liver, an area where substantial CYP450-mediated xenobiotic metabolism occurs, appears to be the main target of cylindrospermopsin toxicity and where cylindrospermopsin and its metabolites bind to proteins (Runnegar et al. 1995; Shaw et al. 2000, 2001; Norris et al., 2001).

Animal studies evaluating the elimination of cylindrospermopsin in urine and feces after i.p. exposures found a continued urinary and fecal excretion over the monitoring period (24 hours) and a mean total recovery from the urine and feces of 76.9% of the administered dose after 24 hours (Norris et al., 2001). Urinary excretion accounted for 68.4% of the 24-hour total and fecal excretion for 8.5%. There was considerable interanimal variability in this study.

2.5.2 Noncancer Health Effects Data

2.5.2.1 Human Studies

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

Dermal exposure to cylindrospermopsin was evaluated using skin-patch testing in humans (Pilotto et al., 2004; Stewart et al, 2006). Exposed individuals showed mild irritation, but no statistically significant dose-response relationship or reaction rates were found between skin reactions and increasing cell concentrations for either whole or lysed cells (Pilotto et al., 2004). No detectable skin reactions were observed in individuals exposed to lyophilized *C. raciborskii* (Stewart et al., 2006).

2.5.2.2 Animal Studies

Most of the information on the noncancer effects of cylindrospermopsin in animals is from oral and i.p. administration studies in mice exposed to purified compound or extracts of *C. raciborskii* cells. Studies conducted with purified toxin are preferred because extracts may contain other toxins or compounds with similar chemical physical properties that co-elute with the toxin. Effects on the liver and kidney, including changes in organ weights and histopathological lesions, along with increases in the hematocrit level in serum and deformation of red blood cell are observed following short-term and subchronic oral exposure to

cylindrospermopsin (Humpage and Falconer, 2002, 2003; Reisner at al., 2004; Sukenik et al., 2006). Oral and i.p. acute toxicity studies in mice also report histopathological effects in both liver and kidney. No chronic toxicity studies evaluating cylindrospermopsin are available.

No oral reproductive or developmental studies are available for cylindrospermopsin. Developmental toxicity studies following i.p. administration of cylindrospermopsin provide some evidence for maternal toxicity and decreased postnatal pup survival and body weight (Rogers et al., 2007; Chernoff et al., 2011). Sibaldo de Almeida et al. (2013) did not find any visceral or skeletal malformations in the offspring of pregnant rats receiving an oral dose of 3 mg/kg/day purified cylindrospermopsin during gestation (GD 1-20).

2.5.3 Mode of Action for Noncancer Health Effects

2.5.3.1 Liver

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

2.5.3.2 Red Blood Cells

There was evidence of effects on red blood cells (RBCs) in the Reisner et al. (2004) and Humpage and Falconer (2002) studies of purified cylindrospermopsin. In the Reisner et al. (2004) report, microscopic examination of blood samples showed the presence of RBCs with spiked surfaces rather than their normal biconcave-disc shape. The authors attributed the acanthocyte formation to an increase in the cholesterol to phospholipid ratio of the RBC membrane. Phospholipids constitute the matrix material of cell membranes. The authors hypothesized that this change was the consequence of decreased activity of plasma lecithin cholesterol acyl transferase (LCAT), an enzyme associated with high-density lipoproteins and the esterification of plasma cholesterol. Effects on the cholesterol content of the RBC membrane can occur with inhibition of the enzyme increasing membrane fluidity and mean corpuscular volume. Associated effects were observed in the Reisner et al. (2004) and Humpage and Falconer (2002) studies. Removal of the abnormal blood cells by the spleen increases both spleen weight and serum bilirubin as well as stimulates hematopoiesis. Additional research is

needed to examine the LCAT enzyme inhibition hypothesis in order to confirm whether it accounts for the effects on the RBC as a result of cylindrospermopsin exposure.

2.5.3.3 Kidney

No mode of action information for kidney effects was observed in the available studies of cylindrospermopsin. Since all the studies were conducted in mice, a species that excretes low molecular weight proteins in urine, there is a need to conduct a study of cylindrospermopsin in a laboratory species that does not excrete protein in the urine in order to determine whether there are comparable effects on kidney weight, protein excretion and renal cellular damage. Kidney necrosis and a decreased renal failure index at the high cylindrospermopsin doses provide support for the effects on the kidney.

2.5.4 Carcinogenicity Data

No chronic cancer bioassays of cylindrospermopsin were located in the literature. Limited data from an *in vivo* study showed no indication that the cyanobacterial extract containing cylindrospermopsin initiated tumors in mice (Falconer and Humpage, 2001). Cell transformation in Syrian hamster embryo (SHE) cells was observed using purified cylindrospermopsin (Marie et al., 2010). Transformation frequency increased at the lowest concentrations (from 1 x 10^{-2} to 1x10^{-7} ng/mL) but not at the highest concentrations (1 or 1x10^{-1} ng/mL).

Mutagenicity studies (e.g., the Ames Assay) have not observed mutagenic activity of cylindrospermopsin (Sieroslawska, 2013). A few i.p. studies investigating the *in vivo* genotoxicity (DNA damage) from exposure to cylindrospermopsin showed DNA strand breakage in the liver of Balb/c mice (Shen et al., 2002) and covalent binding between DNA and cylindrospermopsin, or a metabolite, in Quackenbush mouse liver (Shaw et al., 2000). *In vitro* mutagenic and genotoxic cell assays have shown potential damage to DNA expressed as an increase in micronucleated binucleate cells (MNBNC) in the colon adenocarcinoma line and the human hepatoma line (Bazin et al., 2010), in the human lymphoblastoid cell line (Humpage et al., 2000), in HepG2 cells (Straser et al., 2011), and in isolated human peripheral lymphocytes (Zegura et al., 2011). DNA breaks also have been observed in primary hepatocytes by comet assay (Humpage et al., 2005).

2.6 Conceptual Model

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 cylindrospermopsin 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.

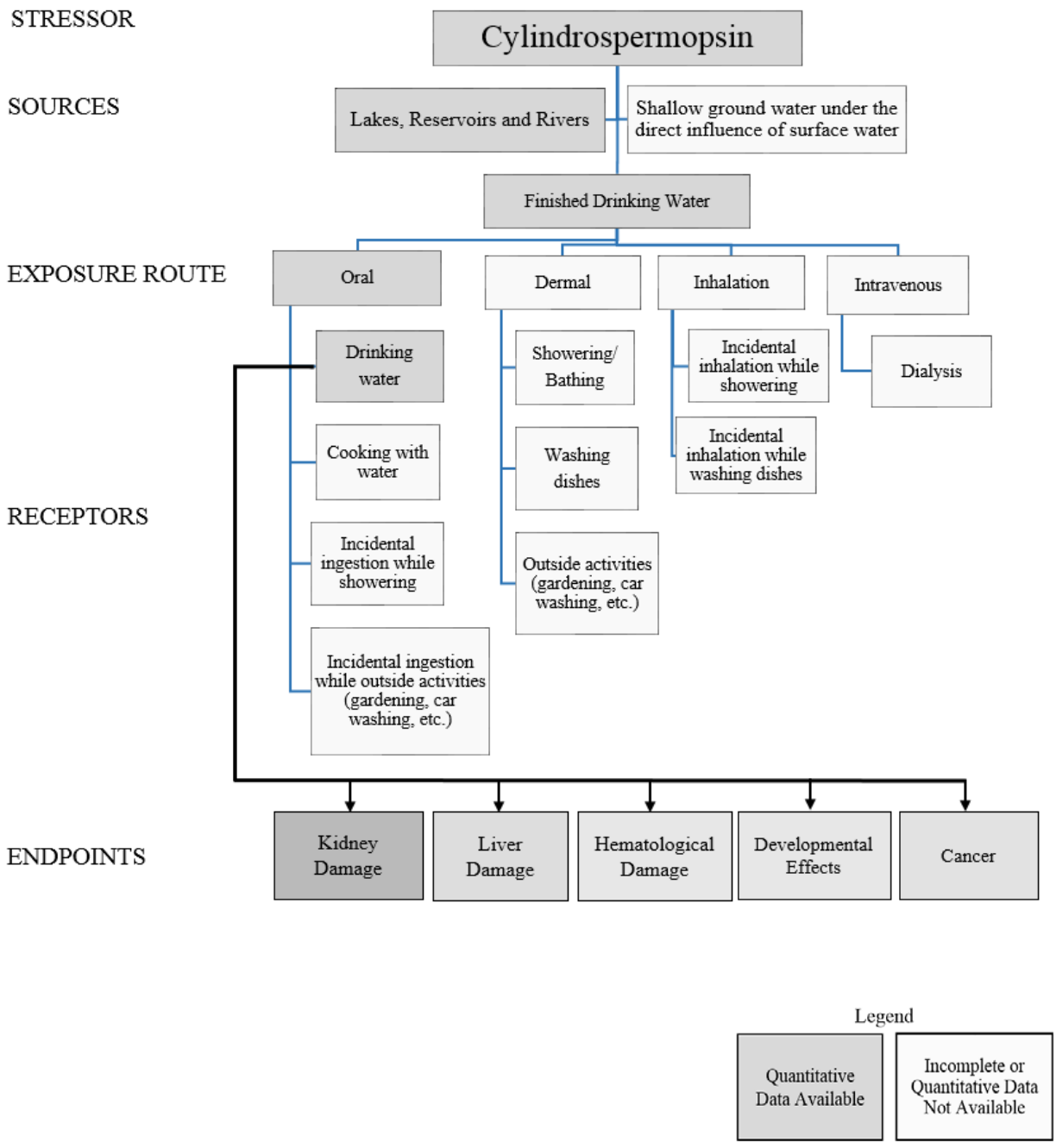
Assessment endpoints for HAs can be developed for both short-term (one-day and ten-day) and lifetime exposures periods using information on the non-carcinogenic and carcinogenic toxicological endpoints of concern. Where data are available, endpoints will reflect susceptible and/or more highly exposed 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 cylindrospermopsin are depicted in the conceptual diagram below (Figure 2-3).

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



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 cylindrospermopsin-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.

2.6.2 Factors Considered in the Conceptual Model for Cylindrospermopsin

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

Sources: Sources of cylindrospermopsin include potential sources of drinking water such as rivers, reservoirs and lakes in the U.S. where blooms producing cylindrospermopsin occur. Shallow private wells under the direct influence of surface water (in hydraulic connection to a surface water body) can also be impacted by cylindrospermopsin-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 with water containing toxins during bathing or showering, washing dishes or outside activities); inhalation exposure (during bathing or showering); 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 cylindrospermopsin because they consume more water per unit body weight than adults. Other individuals of potential sensitivity include 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 on 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, and possibly cylindrospermopsin, were not removed by treatment of dialysis water (Carmichael et al., 2011), identify dialysis patients as a population of potential concern in cases where the drinking water source for the clinic is contaminated with cyanotoxins. EPA has

data to quantify risk to infants, children, and adults based on variability in potential exposure (body weight and drinking water intake rate). However, data are not available to quantify risk to pregnant woman, nursing mothers, persons with liver or kidney disease, or dialysis patients. 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 cylindrospermopsin 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 cylindrospermopsin are limited, but have shown effects on the liver following potential exposure to cylindrospermopsin. Acute, short-term and subchronic studies in animals show effects on the liver, RBC and kidney. In addition, some studies suggest that cylindrospermopsin may lead to reproductive and developmental effects; however, these data are limited. *In vitro* mutagenic and genotoxic cell assays with cylindrospermopsin have shown varied results with some indications of potential damage to DNA. However, these data are limited, and there has been no long term bioassay of purified cylindrospermopsin. Thus, available data are inadequate to assess the carcinogenic potential of cylindrospermopsin at this time. Available toxicity data are described in the *Health Effects Support Document (HESD) for Cylindrospermopsin* (U.S. EPA, 2015a). Kidney effects were selected as the endpoint on which to base the measure of effect. Liver and hematological effects were not as sensitive as the reported kidney effects.

2.7 Analysis Plan

The *Health Effects Support Document (HESD) for Cylindrospermopsin* (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 cylindrospermopsin, 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 cylindrospermopsin was included. The literature search included the following terms: cylindrospermopsin, 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 cylindrospermopsin 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*

- ILS (2000) *Cylindrospermopsin [CASRN 143545-90-8] Review of Toxicological Literature*

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 cylindrospermopsin, 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:

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

Where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (mg/kg bw/day) from a study of an appropriate duration (up to 7 days and 7-30 days for the One-day and Ten-day HAs, respectively).

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 (BMD) 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 cylindrospermopsin; and the potential Mode of Action (MOAs), or toxicity pathways related to the health effects identified.

3.1 Dose-Response

3.1.1 Critical Study Selected

The critical study selected for the derivation of the reference dose (RfD) for cylindrospermopsin is Humpage and Falconer (2002, 2003). Humpage and Falconer (2002, 2003) is a comprehensive toxicity study in which male mice were exposed by gavage to purified cylindrospermopsin from cell extract for 11 weeks. The study authors used four dose groups, adequate numbers of animals per dose group (10) and evaluated a variety of endpoints. Statistically significant, dose-related effects on the kidney, liver and serum chemistry were observed. The kidney was the most sensitive target of toxicity. The Humpage and Falconer (2002) data are supported by the short-term Reisner et al. (2004) results showing exposure-duration-related increased kidney weights, liver weights and testes weights, and hematological effects (acanthocytes or abnormal red blood cells (RBCs) and changes in hematocrit) following a 21-day exposure.

Purified cylindrospermopsin in water was administered by gavage in doses of 0, 30, 60, 120 or 240 μg/kg/day to groups of male Swiss albino mice (6 to 10 mice per dose group) for 11 weeks (Humpage and Falconer, 2002, 2003). The cylindrospermopsin was from an extract of freeze-dried *C. raciborskii* cells Woloszynska (AWT 205) purified using sephadex size-exclusion gel (G-10). The individual sephadex fractions were assayed using high-performance liquid chromatography (HPLC) and concentrated to a sample that was 47% cylindrospermopsin by dry weight and 53% phenylalanine. Food and water consumption, and body weight were examined throughout the study. After 9 weeks of exposure, the study authors report conducting a clinical examination to detect physiological and behavioral signs of toxicity but do not specify the parameters evaluated. Hematology evaluations (4 to 5 per dose group, except the high dose), serum chemistry (4 to 6 per dose group), and urinalysis (6 or 10 per dose group) were conducted. All the evaluations were conducted either near or at the end of the treatment period.

Postmortem examinations were done on the following organs: liver, spleen, kidneys, adrenal glands, heart, testes, epididymis and brain, including measurement of organ weights. Comprehensive histological evaluations were conducted in accordance with the recommendations from the Organization for Economic Cooperation and Development (OECD).

No deaths or visual clinical signs of toxicity were reported in mice exposed to purified cylindrospermopsin under the study conditions. The mean final body weight was 7-15% higher in all dose groups compared to controls, but was not dose-related and was only statistically significant at 30 and 60 µg/kg/day (Humpage and Falconer, 2003). No significant changes were observed in food consumption. In all dose groups, the water intake was significantly reduced; water consumption was 53% of the control level at 30 µg/kg/day and the higher dose groups were 68-72% of the control levels.

Relative kidney weight was significantly increased in a dose-related manner at ≥60 µg/kg/day (12-23% greater than controls; see Table 3-1). Relative liver weight was significantly increased (13% greater than controls) only at the highest dose (240 µg/kg/day). Relative spleen, adrenal and testes weights were increased for doses ≥60 µg/kg/day, but the differences from control were not statistically significant (Humpage and Falconer, 2002).

Selected serum chemistry (n= 4-6), hematology (n=4-5) and urinalysis (n=6-10) results are shown in Table 3-2. The hematology and serum chemistry evaluations showed no dose-related, statistically significant changes, although serum albumin, total bilirubin and cholesterol were increased compared to controls at all doses (Humpage and Falconer, 2002). The increases in cholesterol were significant for the 30 and 60 µg/kg/day groups, but not at the higher doses. The serum urea concentration was slightly decreased at the two highest doses. A nonsignificant increase in red cell polychromasia (high number of RBCs) was indicated for all doses, but quantitative data were not presented. Packed red cell volume was slightly increased and mean corpuscular hemoglobin was slightly decreased (Table 3-2) when compared to controls, although the changes were not dose related. When combined with the bilirubin results and the increased relative spleen weight, the hematological data suggest the possibility of minor RBC effects. One of the limitations in the serum chemistry and hematology data is the small number of samples evaluated, a factor that impacts the determination of statistical significance (Humpage and Falconer, 2002).

There was a significant decrease in the urine protein-creatinine ratio (g/mmol creatinine) at 120 and 240 µg/kg/day compared to the controls (51% and 37% of controls, respectively; both $p<0.001$) (Humpage and Falconer, 2002). Also, a significant decrease in urine specific gravity normalized for creatinine was seen at 240 µg/kg/day compared to the control ($p<0.001$). The renal glomerular filtration rate (GFR) was decreased compared to controls at all doses, but the differences were not dose dependent or statistically significantly different from controls. The renal failure index[1] was decreased slightly at ≥120 µg/kg/day; the differences from control were not statistically significant (Humpage and Falconer, 2002). Tubular retention of low molecular weight urinary proteins could account for the decreased urinary protein and possibly the increased kidney weight. Although effects on kidney weight and urine protein levels were observed in male mice, the biological relevance of the latter effect and whether it would also occur in female mice needs further investigation. Mice are known to excrete a group of functional, highly-polymorphic, low-molecular-weight urinary proteins that play important roles in social recognition and mate assessment (Cheetham et al., 2009). The relevance of the urinary protein findings in mice to humans is unknown.

[1] Renal failure index= (urinary sodium concentration × plasma creatinine concentration) / urinary creatinine concentration

Table 3-1. Kidney Weight Data from Oral Toxicity Study of Cylindrospermopsin Administered Daily over Eleven Weeks (Humpage and Falconer, 2002, 2003)

Dose (µg/kg/day)	Number	Relative Kidney Weight		% Difference	Significance
		Control g/100g $_{BW}$	Exposed g/100g $_{BW}$		
0 (Control)	10	1.48	-	-	-
30	10	1.48	1.57	+6	Not significant
60	9	1.48	1.66	+12	p <0.001
120	9	1.48	1.82	+23	p <0.001
240	6	1.48	1.78	+20	P <0.001

Table 3-2. Selected Clinical Chemistry, Hematology and Urinalysis Findings (Humpage and Falconer, 2002, 2003)

Endpoint	N	Dose (µg/kg/day)				
		0	30	60	120	240
Clinical Chemistry						
Urea (mmol/L)	4-6	9.24	9.22	8.55	7.51	7.92
Albumin (g/L)	4-6	23.8	26.6	26.0	26.0	25.8
Cholesterol (mmol/L)	4-6	3.26	4.60**	4.65**	3.68	4.08
Bilirubin (mmol/L)	4-6	2.62	2.72	2.88	3.06	3.07
Hematology						
Packed Cell volume (L/L)	4-5	0.38	0.39	0.39	0.39	ND
Mean Corpuscular Hemoglobin (MCH, pg/L)	4-5	16.8	15.7	16.4	16.4	ND
Urinalysis						
Volume (mL)	6-10	9.85	11.18	10.38	11.74	6.74
Creatinine (mmol/L)	6-10	0.57	0.49	0.54	0.51	0.72**
Specific gravity/creatinine	6-10	1.79	2.04	1.91	1.99	1.44*
Protein/creatinine (g/mmol)	6-10	4.3	3.6	3.3	2.2**	1.6**
Renal Failure Index (mmol/L)	4-6	4.3	4.3	4.5	3.6	3.6

ND = not determined
Significantly different from control: *p<0.05; **p<0.01.

Serum albumin and total serum protein were not decreased in the Humpage and Falconer studies (2002, 2003). The most sensitive effects observed by Humpage and Falconer (2002, 2003) were dose-related decreases in the urinary protein: creatinine ratio at ≥120 µg/kg/day and increased relative kidney weight at ≥60 µg/kg/day. The noted decrease in urinary protein excretion could reflect an impact on excretion of mouse urinary proteins given the fact that total serum protein was not significantly increased compared to controls for all dose groups. Mouse urinary proteins are synthesized in the liver (Clissold and Bishop, 1982) and transported to the kidney for excretion. If cylindrospermopsin did reduce liver protein synthesis, a decrease in total serum protein would be expected. However, this was not the case, suggesting a lack of an effect on synthesis of the urinary proteins in the liver.

The Humpage and Falconer (2002, 2003) postmortem tissue examinations showed histopathological damage to the liver based on scores assigned for necrosis, inflammatory foci and bile duct changes at ≥120 µg/kg/day. The percent of animals with liver lesions in the 120 and 240 µg/kg/day dose groups was 60% and 90%, respectively, when compared to 10%, 10% and 20% for the 0, 30, and 60 µg/kg/day dose groups, respectively. Severity scores were not given, and the liver lesions were not further described. There was proximal renal tubular damage in kidney sections from two mice in the 240 µg/kg/day dose group (Humpage and Falconer, 2002, 2003).

The 11-week study by Humpage and Falconer (2002, 2003) provides a NOAEL (30 µg/kg/day) and a LOAEL (60 µg/kg/day) for dose-related, statistically significant increases in kidney weights along with indicators of reduced renal function effects at higher doses. Because of the similarity in the type of effects observed and the LOAELs from the Humpage and Falconer (2002, 2003) and Reisner et al. (2004) studies, the selection of the NOAEL from Humpage and Falconer was determined to be the most appropriate point of departure for ten-day exposures in infants, children and adults despite its longer exposure duration.

3.1.2 Endpoint Selection

Upon considering all effects observed by Humpage and Falconer (2002, 2003), increased relative kidney weight was considered the most appropriate basis for quantitation. Adverse effects on the kidneys were manifested by decreases in urinary protein concentration and increased relative kidney weight. The study authors reported significantly increased relative kidney weight at ≥60 µg/kg/day, decreased urinary protein and liver lesions at ≥120 µg/kg/day and renal tubular lesions at 240 µg/kg/day (Humpage and Falconer, 2002, 2003). Relative kidney weight increased significantly in a dose-related manner beginning at 60 µg/kg/day (12-23% greater than controls), and relative liver weight was significantly increased at 120 µg/kg/day (12-23% greater than controls) and at the high dose of 240 µg/kg/day (13% greater than controls). Relative spleen, adrenal and testes weights were increased for doses ≥60 µg/kg/day, but the differences from control, although dose-related, were not statistically significant. Humpage and Falconer (2002, 2003) identified the LOAEL as 60 µg/kg/day and the NOAEL as 30 µg/kg/day based on the dose-related and statistically significant increase in relative kidney weight. These adverse effects are potential indicators of suppressed hepatic protein synthesis that was not

reflected in the measurement of total serum protein and/or increased retention of low molecular weight mouse urinary proteins by the kidney because of damage to the renal tubules.

In the single dose drinking water study by Reisner et al. (2004), hematological effects (acanthocytes, increased hematocrit) and increased organ weights (liver, testicular and kidney) in young (4 week) male Institute for Cancer Research (ICR) mice were observed following a three week exposure to purified cylindrospermopsin. The 66 µg/kg/day LOAEL is comparable to that from the Humpage and Falconer (2002, 2003) study (60µg/kg/day). Humpage and Falconer (2002, 2003) evaluated 5 different doses using 6 to 10 mice per dose group; Reisner et al. (2004) used one dose with 8 male mice. Reisner et al. (2004) demonstrated effects in comparable parameters to those impacted in Humpage and Falconer at a dose of 66 µg/kg/day with a three week exposure. They also demonstrated a trend for effects on kidney weight and hematocrit across the three-week duration of exposure. Because the renal effects reported in Humpage and Falconer (2002, 2003) did not occur at 11 weeks for the 30 µg/kg dose, the point of departure from the Humpage and Falconer study was determined to be the most appropriate for the quantitative assessment. Thus, the quantification from the Humpage and Falconer NOAEL based on kidney weight changes provides the best point of departure for ten-day exposures in children and adults despite its longer exposure duration.

3.2 Ten-Day Health Advisory

The Ten-day HA is considered protective of non-carcinogenic adverse health effects over a ten-day exposure to cylindrospermopsin 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:

$$\text{Ten-day HA} = \frac{30\,\mu\text{g/kg/day}}{300 \times 0.15\,\text{L/kg/day}} = 0.7\,\mu\text{g/L}$$

Where:

30 µg/kg/day	=	The NOAEL for kidney effects in mice exposed to cylindrospermopsin in water for 11 weeks (Humpage and Falconer, 2002, 2003).
300	=	The composite uncertainty factor (UF) including a 10 for intraspecies variability (UF_H), a 10 for interspecies differences (UF_A), and a 3 for uncertainties in the database (UF_D).
0.15 L/kg/day	=	Normalized drinking water intakes 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 0.7 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 cylindrospermopsin 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:

$$\text{Ten-day HA} = \frac{30\,\mu\text{g/kg/day}}{300 \times 0.03\,\text{L/kg/day}} = 3\,\mu\text{g/L}$$

Where:

30 µg/kg/day	=	The NOAEL for kidney effects in mice exposed to cylindrospermopsin in water for 11 weeks (Humpage and Falconer, 2002, 2003).
300	=	The composite UF including a 10 for intraspecies variability (UF_H), a 10 for interspecies differences (UF_A), 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 3 µg/L is considered protective of non-carcinogenic adverse health effects for children of school age through adults over a ten-day exposure to cylindrospermopsin 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 a low RBC count as a result of genetic or nutritional factors could be more sensitive to cylindrospermopsin exposures than the general population. Individuals with pre-existing kidney/liver problems may also be more sensitive. Pregnant woman, nursing mothers, and the elderly could also be sensitive to cylindrospermopsin 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 cylindrospermopsin. Allometric scaling is not applied in the development of the Ten-Day HA values for cylindrospermopsin. 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_D - An uncertainty factor of 3 ($10^{0.5} = 3.16$) is selected to account for deficiencies in the database for cylindrospermopsin. The database for cylindrospermopsin includes limited human studies. The database for studies in laboratory animals includes oral exposure acute, short-term and subchronic studies, but many of them lacked a comprehensive evaluation of a wide spectrum of effects. The database lacks chronic toxicity and multi-generation reproductive and developmental toxicity studies using the oral route of exposure. There is a lack of data on neurological and immunological endpoints. The RBC parameters evaluated differed between the Humpage and Falconer (2002, 2003) and Reisner et al. (2004) studies.

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., $10^{½}$) 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 Studies Supporting Determination of Critical Study

Increases in kidney weight and hematological effects are detected in all three studies (Humpage and Falconer, 2002, 2003; Reisner et al., 2004; and Sukenik et al., 2006). However, the type of hematological effects varied among studies as did the statistical significance of the observed effects. Humpage and Falconer (2002, 2003) found signs indicative of hemolysis (increased bilirubin, spleen weight and polychromasia (high number of RBCs with low hemoglobin)), while Reisner et al. (2004) and Sukenik et al. (2006) found acanthocytes (abnormal RBCs). Increases in kidney weight were significant for Humpage and Falconer (2002, 2003) and Sukenik et al. (2006), but not statistically significant for Reisner et al. (2004). Humpage and Falconer (2002, 2003) and Reisner et al. (2004) used purified cylindrospermopsin, while Sukenik et al. (2006) used an extract in spent medium. Of these three studies, Humpage and Falconer (2002, 2003) provides a NOAEL (30 µg/kg/day) and a LOAEL (60 µg/kg/day) for dose-related statistically significant increases in kidney weights and indications of renal function effects at higher doses. Although the percent change in kidney weight is the same for Reisner et al. (2004), only the change observed by Humpage and Falconer (2002, 2003) was statistically significant.

4.2 Study Duration

The short-term studies with appropriate durations (typically 7 days up to 30 days) available for cylindrospermopsin (Shaw et al., 2001; Reisner et al., 2004), are not suitable for quantification, as described below. However, the Reisner study does support the use of the Humpage and Falconer (2002, 2003) study for the derivation of the Ten-day HA, despite the longer duration of the study.

The Shaw et al (2001) study reported the results from multiple experiments. These experiments each have limitations including use of extract, lack of adequate numbers of animals and monitored endpoints, and the limited number of doses tested that preclude their use in quantification. The oral data for purified extract from Shaw et al. (2001) identified fatty liver as an adverse effect in mice following a 14 day gavage exposure to 0.05 mg/kg/day. However, the only effects mentioned in the published paper are the liver effects and an absence of lymphophagocytosis in the spleen.

Reisner et al. (2004) conducted a 21 day study in mice and showed significant increases in hematocrit, acanthocytes (abnormal RBCs), and liver and testes weights effects at a 66 µg/kg/day dose and a duration-related nonsignificant increase in and kidney weight. This study was not selected for development of the Ten-day HA because this study used a single dose; however, the effects to that dose after 3-weeks were comparable to the effects seen in the

Humpage and Falconer (2002, 2003) study at a slightly lower 60 mg/kg/day dose after 11 weeks. The Humpage and Falconer (2002, 2003) study was determined to be the most appropriate for the quantitative assessment because the LOAEL at 11 weeks would be protective for the effects seen at 3-weeks in the shorter duration study.

4.3 Allometric Scaling Approach

Allometric scaling was not applied in the development of the RfD for cylindrospermopsin. 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 development 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.

4.4 Uncertainty and Variability

Uncertainty factors were applied in several areas to adjust for incomplete information. Human data on the toxic effects of cylindrospermopsin are limited. Quantification for the absorption, distribution and elimination of cylindrospermopsin 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 increased kidney weight, decreased urinary protein levels, decrease in renal failure index and the formation of acanthocytes (abnormal RBCs) is not known. In animal studies with cylindrospermopsin, adverse effects (RBC effects) observed 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 characterized in the human population following exposure to cylindrospermopsin. Additional information is needed on the potential health risks from mixtures of cylindrospermopsin with other cyanotoxins, bioactive molecules with an effect on living organisms and chemical stressors present in ambient water and/or drinking water supplies. The critical study was conducted only in male mice and therefore, any gender-specific effects of cylindrospermopsin are not understood.

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

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

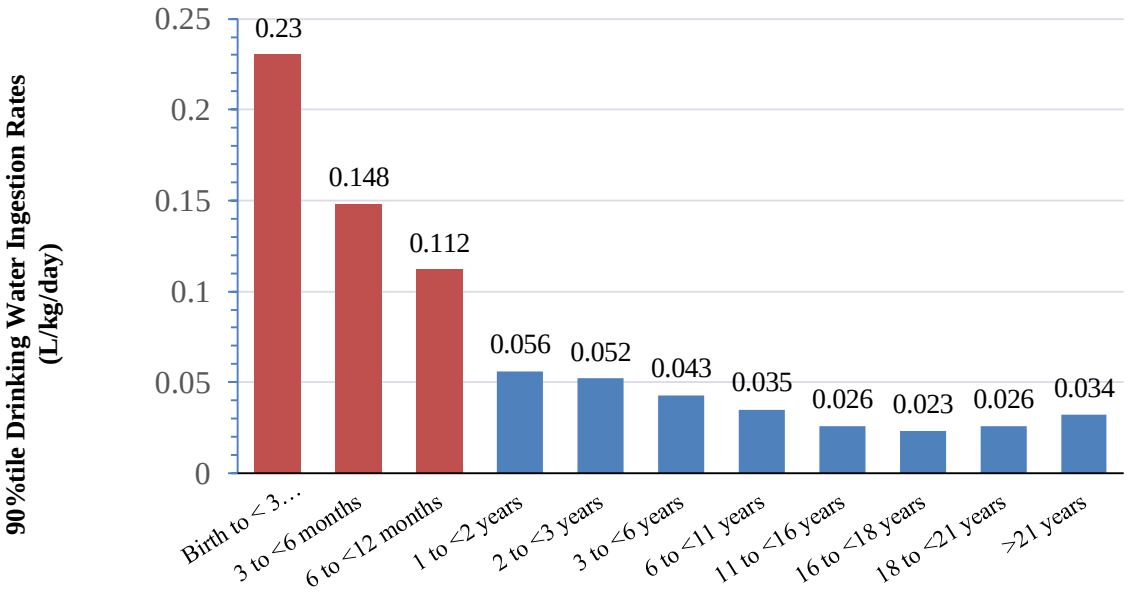
Based on the currently available science, evidence is lacking to assess differences in susceptibility between infants, children and adults. There are, however, significant differences in exposure between these life-stages that impact risk.

4.6 Distribution of Body Weight and Drinking Water Intake by Age

Both body weight and drinking water intake distributions vary with age. EPA has developed two health advisory values, a Ten-day HA of 0.7 µg/L based on exposure to infants over the first year of life, and a Ten-day HA of 3 µg/L based on exposure to adults, over 21 years of age. Section 4.7 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 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).

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).

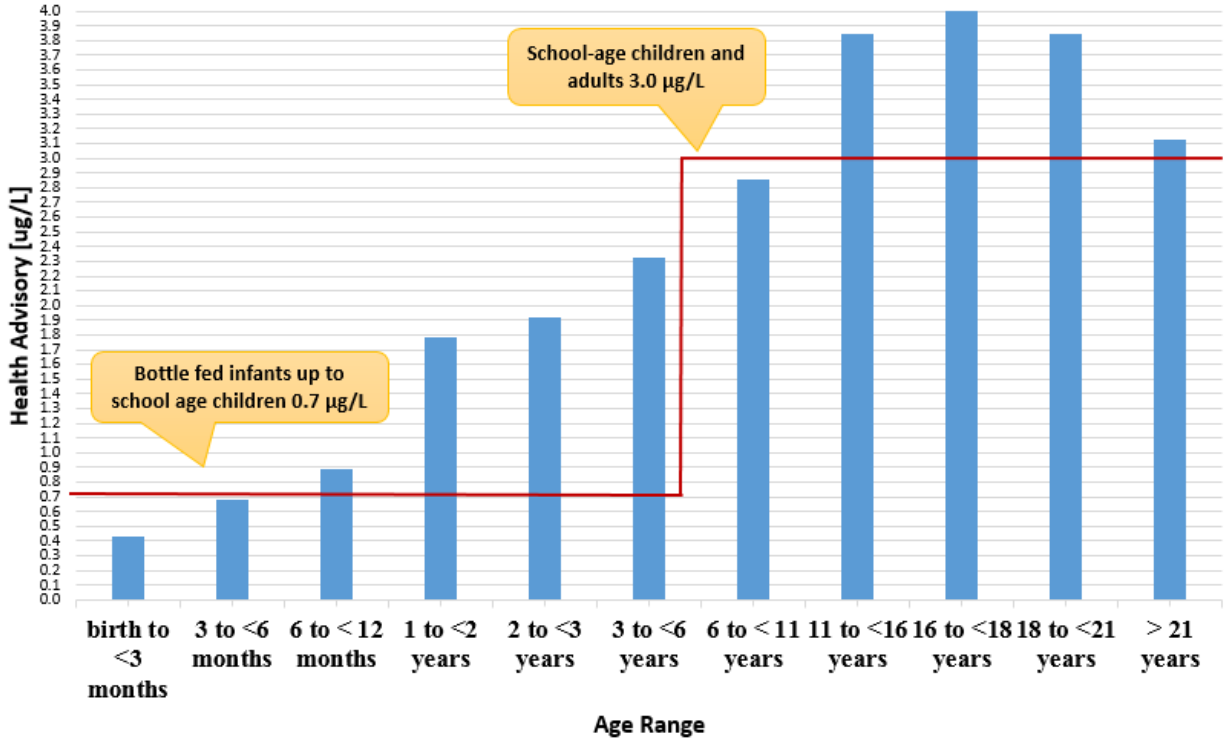
Based on the drinking water intake rates for children <12 months (0.15 L/kg-day), the exposure of children is over 4 times higher than that of adults >21 years old on a body weight basis (0.034 L/kg-day). Infants from birth to 3 months may be exclusively bottle-fed and therefore, have a higher ingestion 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 cyanotoxins 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 cylindrospermopsin through breast milk are not available for humans.

4.7 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 cylindrospermopsin for each age group (plotted in Figure 4-2) to demonstrate the variability due to body weight and drinking water intake by age.

EPA decided to apply the Ten-day HA value calculated for infants over the first year of life (0.7 µg/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

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



months old is 0.4 µg/L, slightly below the infant health advisory value of 0.7 µg/L. EPA believes that infants from birth to 3 months old are not at a disproportionate risk at a 0.7 µg/L advisory value because a safety factor of 30 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 0.7 µg/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 3 µg/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

The primary methods used for the analysis of cylindrospermopsin are liquid chromatography (LC) and enzyme linked immunosorbent assay (ELISA). Several detection modes are generally coupled with LC including single channel ultraviolet (UV)/visible and multi-channel UV photodiode array (PDA), electrospray ionization mass spectrometry (LC-ESI/MS), and electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS). Due to the limited selectivity of UV-based detectors, the use of mass spectrometric detection is becoming more commonplace. Commercial ELISA test kits are also available for cylindrospermopsin detection. These kits are available in both semiquantitative and quantitative formats and are easily adapted to field or "screening" measurements.

EPA has recently released Method 545 (U.S. EPA, 2015c) which is a LC-ESI/MS/MS method for the determination of cylindrospermopsin and anatoxin-a in drinking water. This method requires the operation of the mass spectrometer in MS/MS mode to enhance selectivity. In this method, samples are preserved with ascorbic acid (dechlorinating agent) and sodium bisulfate (microbial inhibitor). In the laboratory, aliquots (1 mL) of sample are taken for analysis, and internal standards are added. An aliquot of the sample is injected into an LC equipped with an analytical column that is interfaced to the mass spectrometer. The analytes are separated and then identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical liquid chromatography tandem mass spectrometry (LC-MS/MS) conditions. The concentration of each analyte is determined using the integrated peak area and internal standard technique. A single laboratory lowest concentration method reporting limit (LCMRL) of 0.063 µg/L was determined for cylindrospermopsin along with an average value of 0.083 µg/L for all participants of a multi-lab evaluation (n=4) (Winslow et al., 2006). Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI/MS/MS) is available at http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods_ogwdw.cfm.

Other LC methods have generally used UV spectroscopic detection for cylindrospermopsin analysis. These methods have often incorporated solid phase extraction (SPE) to preconcentrate the target analyte, reduce matrix interferences or both. Quantitation limits have ranged from 4 µg/L to < 0.1 µg/L based on the instrumental setup and the use of preconcentration steps (Papageorgiou et al., 2012).

Commercial ELISA kits for the detection of cylindrospermopsin are available from several vendors. These kits claim a working concentration range of 0.05 and 2 µg/L.

6.0 TREATMENT TECHNOLOGIES

The information below is adapted from the draft 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 *International Guidance Manual for the Management of Toxic Cyanobacteria* (GWRC, 2009) and *Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities* (Newcombe et al., 2010) available at: http://www.waterra.com.au/cyanobacteria-manual/PDF/GWRCGuidanceManualLevel1.pdf and 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 from 5,000 to 15,000 cells/mL) (WHO, 1999), are less likely to release significant cyanotoxin concentrations upon cell lysis and may mitigate or prevent a cyanobacterial bloom from proliferating as the season progresses. If a cyanobacteria bloom does occur, utilities may investigate alternative raw water sources, change intake locations or levels to withdraw raw 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). These strategies can be applied to the entire source water body or to just a portion of the lake depending on the need, size and depth of the water body relative to the source water intake(s).

The use of ultrasonic sound waves, or sonication, to disrupt cyanobacterial cells has also been investigated as a potential source water treatment option (Rajasekhar et al., 2012). 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. The water treatment methods need to be tailored to the type(s) of cyanobacteria present, the site-specific water quality (e.g., pH, temperature, turbidity, presence of natural organic material (NOM)), the treatment processes already in place and multiple treatment goals (e.g., turbidity and total organic carbon (TOC) removal, disinfection requirements, control of disinfection by-products (DBP) formation). 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 intracelluar toxins. Care should be taken to avoid cell lysis. A multi-barrier approach consists 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.

When released from the cell, cylindrospermopsin can be found dissolved or attached to other materials such as particulate or soluble substances. Powdered activated carbon (PAC) has proven to be effective for removal of extracellular cylindrospermopsin. Limited information is available on the adsorption of cylindrospermopsin onto granular activated carbon (GAC).

6.2.1 Conventional Treatment for Cylindrospermopsin

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. Rapid sand filtration without pre-treatment (i.e., direct filtration, without coagulation/clarification) is not effective for cyanobacterial cell removal.

Conventional water treatment (coagulation, flocculation, sedimentation or dissolved air flotation (DAF), and filtration) is considered effective for removal of intracellular toxins but ineffective for dissolved cyanotoxins such as cylindrospermopsin, which is partially dissolved in water under normal growth conditions (Chow et al., 1999; Rapala et al., 2006; Carrière et al., 2010). Application of a multiple barrier approach has the potential to be effective (Newcombe et al., 2015). Ho et al. (2008, 2011) conducted bench-scale studies and modeling on the use of PAC for the adsorption of cylindrospermopsin. The results demonstrated that a PAC dose of 25 mg/L and a contact time of 60 minutes would be required to reduce 5 µg/L of cylindrospermopsin to less than 1 µg/L. When concentrations of cylindrospermopsin are 1-2 µg/L or 3-4 µg/L, the recommended doses of PAC are 10-20 mg/L and 20-30 mg/L, respectively (Newcombe et al., 2010).

Dixon et al. (2011b) also conducted laboratory-scale testing of integrated membrane systems for cyanotoxin removal. The results showed that an ultrafiltration system with pre-treatment using 2.2 mg/L of alum and 20 mg/L of PAC resulted in 97% removal of intra- and extra-cellular cylindrospermopsin to achieve a treated water concentration of less than 0.1 µg/L (Dixon et al., 2011a). Nanofiltration and reverse osmosis would likely be effective in removing dissolved toxins, but only a few studies have been conducted. Dixon et al. (2011a) studied the removal of cyanobacterial toxins by nanofitration and found that average removals between 90-100% could be achieved for cylindrospermopsin using membranes with a low molecular weight cut-off (MWCO) (< 300 Daltons).

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 cyanotoxins. Extracellular cylindrospermopsin may be removed by many treatment plants using existing treatments such as chlorination or by the addition of PAC (Carriere et al., 2010). Although it is possible to remove both intracellular and extracellular toxins effectively using a combination of treatment processes, the removal efficiency can vary considerably. Utilities need to ensure that they are using their existing treatment processes to their fullest capacity for removal of both cyanobacterial cells and extracellular toxins, and that the appropriate monitoring is being conducted to ensure that adequate removal is occurring at each step in the treatment process.

6.2.2 Chemical Oxidation

Chemical oxidation using chlorine or ozonation can be effective at oxidizing cylindrospermopsin, but can also cause the cells to lyse, resulting in an increase in concentrations of extracellular toxins in drinking water. By applying conventional filtration (or another filtration process) first to remove the majority of intact cells, the extracellular cylindrospermopsin 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).

Different cyanotoxins react differently to oxidants depending on the individual characteristics of the source water such as TOC, temperature and pH (Westrick et al., 2010). While chlorination is an effective treatment for oxidizing cylindrospermopsin, its effectiveness is dependent on pH. Rodriguez et al. (2007) found that at a pH of 7 and an initial chlorine dose of 1 mg/L, oxidation of cylindrospermopsin is fast, with almost complete reaction after 30 minutes. Other chlorinated oxidants such as chloramines and chlorine dioxide have little impact on cylindrospermopsin due to a slow reaction rates. For example, the reaction of chlorine dioxide with cylindrospermopsin is relatively slow with a second-order rate constant of 0.9 $M^{-1}s^{-1}$ at pH 8. The rate constant is pH-dependent and decreases significantly under mildly acidic conditions. Chlorine dioxide may be used to inactivate *C. raciborskii*, however, in typical drinking water treatment applications, it does not appear to be practical for oxidizing cylindrospermopsin given its slow reaction rate (de la Cruz et al., 2013). Oxidation by potassium permanganate is temperature dependent and has not been shown to be effective in oxidizing cylindrospermopsin. Water treatment utilities that use chloramines or chlorine dioxide as disinfectants to reduce the formation of regulated disinfection by-products may want to reconsider oxidation efficacy for cyanotoxin inactivation during periods when algal toxins are present in source waters, while balancing these other treatment objectives. Ozone has been shown to effectively oxidize cylindrospermopsin in laboratory-scale studies (de la Cruz et al., 2013). At pH 8, approximately 95% of cylindrospermopsin (initial concentration of 415 µg/L) was oxidized using 0.38 mg/L O_3.

6.2.3 Ultraviolet Irradiation

Studies have indicated that ultraviolet (UV) irradiation may be effective for the oxidation of cylindrospermopsis cells (Westrick et al., 2010). However, exposure times and/or UV doses tested in the bench-scale experiments were greater than those typically applied in drinking water treatment.

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 cyanotoxins. At this time, no units have been evaluated for removal of cylindrospermopsin. Further studies need to be conducted to assess the efficacy of home filtration devices for various cyanotoxins, including cylindrospermopsin, and for other filtering conditions such as increased toxin load and the presence of other contaminants in drinking water. 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/.

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