Factors Contributing to Cyanobacteria Blooms and Cyanotoxin Production and Release

AUGUST 19, 2021

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Acronyms

ATX	anatoxin-a
CYN	cylindrospermopsin
DAF	dissolved air flotation
dhATX	dihydroanatoxin-a
EPS	extracellular polysaccharides
fg	femtogram
GAC	granular activated carbon
HAB	harmful algal blooms
MC	microcystin
NOD	nodularin
PAC	powdered activated carbon
RuBisCo	ribulose bisphosphate carboxylase/oxygenase
SRP	soluble reactive phosphate
STX	saxitoxins
TN	total nitrogen
TP	total phosphorus
USEPA	United States Environmental Protection Agency

Executive Summary

ToxStrategies conducted a comprehensive literature review on the factors and conditions causing and contributing to cyanobacterial blooms, as well as their toxin production and release, especially as they relate to Texas surface waters. This report discusses the various environmental factors and their complex relationships with cyanobacteria and their toxins. However, environmental factors discussed are general in nature, and there can be significant variability among waterbodies, even within the same region. Drinking water treatment methods and Texas case studies are presented as well.

Cyanobacteria, also known as "blue-green algae," occur in surface waters worldwide. Cyanobacterial toxins, or "cyanotoxins," released from cyanobacteria can harm the health of animals and humans. Cyanobacterial blooms are typically associated with eutrophication, which is enhanced biological production in surface waterbodies as a result of increased nutrient loads. When cyanobacteria in these bloom events lyse, they release their cyanotoxins. Cyanobacteria have been found throughout water columns as planktonic species, where they can aggregate during blooms, as well as in the benthos as mats. Historically, they have been considered as algae, given their morphologies. However, morphology alone is insufficient to identify cyanobacteria, and phylogenetic molecular techniques have become the standard for identification. Improved and expanded phylogenetic profiling continues with the evolution of cyanobacterial taxonomy.

Nutrient concentrations are important environmental factors that promote cyanobacterial growth. Nutrients, namely phosphorus and nitrogen, enter a waterbody from point sources, such as discharges and outfalls, and nonpoint sources, such as surface runoff and groundwater inflow. Phosphorus is frequently considered to be rate limiting because many cyanobacteria can fix nitrogen from the atmosphere, but nitrogen concentrations can also affect cyanobacterial growth. Cyanobacteria can use multiple forms of nitrogen, and they can store phosphorus internally to give them an advantage when external phosphorus concentrations are low.

Other important environmental factors include light availability, temperature, waterbody hydrodynamics, pH, and salinity. Some cyanobacteria have lower light requirements than other phytoplankton, and they have multiple pigments that allow them to harvest light at wavelengths unusable to their non-cyanobacteria competitors. Planktonic cyanobacteria contain gas vesicles that make them buoyant and enable them to remain in the euphotic zone. Some, but not all, cyanobacteria thrive at higher temperatures. Many cyanobacteria have overwintering capabilities in which they can bloom in cold temperatures to keep waters turbid or produce akinetes (dormant cells) that germinate when warm temperatures return. Higher temperatures promote thermal stratification in a waterbody and affect its mixing depth, enabling buoyant cyanobacteria to maintain their access to light. In addition, cyanobacteria that grow

slowly tend to favor waterbodies with long residence times. Large cyanobacterial blooms can raise the pH of a waterbody during photosynthesis because they use a variety of methods to take up inorganic carbon, and some cyanobacteria can grow more successfully at higher pH levels than other phytoplankton. Many cyanobacteria are also resistant to osmotic shock and can survive in higher salinities.

Other organisms interact with cyanobacteria. Many cyanobacteria are resistant to grazing by zooplankton because of their toxicity, filament or colony size, or low nutrition. Blooms can also provide a refuge for small fish that consume zooplankton, reducing their grazing pressure. In particular, zebra mussels selectively reject feeding on cyanobacteria when grazing on phytoplankton, thus giving cyanobacteria a competitive advantage. Zebra mussels can also act as a sink and a source of phosphorus as they mobilize iron-bound phosphorus from sediments. Furthermore, as zebra mussels uptake calcium, they affect waterbody chemistry by reducing alkalinity, which may give cyanobacteria an advantage.

Cyanobacterial populations and behaviors can vary with environmental conditions, even at a local scale. Although multiple studies have attempted to tie regional climate and meteorological conditions to cyanobacteria and cyanotoxin production, variability exists even among waterbodies that are near each other.

Cyanobacteria strains that possess the appropriate genes produce cyanotoxins, and they can co-exist with nontoxic strains. Cyanobacteria within a given species can produce multiple toxins. The amount of cyanotoxin in a given strain can also vary, and seasonal patterns of cyanotoxin production fluctuate among waterbodies. It has been hypothesized that environmental conditions may affect whether genes related to cyanotoxin production are upregulated, but this is not yet well understood. Even if nontoxic clones dominate a given bloom, the toxic clones present may still produce toxins in excess of acceptable limits. Nevertheless, in general, factors that affect cyanobacterial growth are also relevant to their effects on cyanotoxin production.

Cyanotoxins may present health concerns to humans as hepatotoxins, dermatoxins, and neurotoxins when they are released from cyanobacterial cells. This release typically follows cell lysis when the toxins enter surface waterbodies that are used for drinking water or recreational activities, resulting in incidental ingestion. When adjusting the drinking water source intake to avoid cyanobacterial blooms is impractical, drinking water must be treated to remove intracellular (intact cyanobacteria) and extracellular cyanotoxins. Conventional treatment methods may remove cyanobacterial cells and extracellular cyanotoxins, although an integrated approach using multiple methods is often preferred. Drinking water treatment efficacy varies among extracellular cyanotoxins depending on their chemical and physical properties. Coagulation, flocculation, sedimentation, and filtration are often effective at removing intact cells but must be optimized for specific blooms. Dissolved air flotation is useful for removing cyanobacteria with low densities and high buoyancy. Membrane filtration can remove

cyanobacterial cells, and nanofiltration and reverse osmosis can also remove cyanotoxins. Because oxidation at the beginning of a drinking water treatment process has the potential to lyse cyanobacterial cells, treatment plant operators should consider physically removing cells prior to oxidation. This consideration is particularly important for chlorination because it can increase disinfection byproduct concentrations when oxidizing organic cyanotoxins. Whereas ultraviolet irradiation at doses typical for conventional pathogen disinfection is not effective for destroying cyanotoxins, it may be a viable option when used in conjunction with hydrogen peroxide to produce hydroxyl radicals. Potassium permanganate and ozone may also degrade certain cyanotoxins. In addition, activated carbon has been demonstrated to remove cyanotoxins, but tests are required for proper powdered activated carbon dosing or granular activated carbon column design.

There have been few peer-reviewed studies specific to cyanobacterial blooms or cyanotoxins in Texas, but cyanobacteria have been observed widely throughout the state. Several regulatory authorities routinely monitor waterbodies for blooms in and around Texas and warn the public accordingly. Case studies in Texas largely involve animal deaths as they suffer neurological or gastrointestinal effects after consuming water or mats contaminated with cyanotoxins or licking cyanobacteria from their fur. These incidents have raised concerns about cyanobacteria occurrences, and municipal authorities must understand the features and patterns specific to each waterbody because environmental factors are complex and dynamic.

1 Introduction

Cyanobacteria, also known as “blue-green algae,” occur in surface waters across the globe. Cyanobacterial toxins, or “cyanotoxins,” released from cyanobacteria are a concern for the health of humans and animals. Cyanobacteria growth produces harmful algal blooms (HABs) that are typically associated with eutrophication, which is enhanced biological production in surface waterbodies as a result of increased nutrient loads. Eutrophication often leads to visible blooms, surface scums, and floating plant mats. When cyanobacteria in these bloom events die and lyse, they release their cyanotoxins. In addition, when this organic matter decays, it can deplete dissolved oxygen in the water, causing secondary fish kills. Cyanobacterial biomass can produce offensive taste and odor compounds, and also can foul beaches, affecting important tourism industries. Other harmful effects arise when cyanobacteria compete with other plankton for nutrients such as diatoms, which are important to fish food webs (Steffen et al., 2014). Herein, we discuss the properties of cyanobacteria, environmental factors associated with cyanobacterial growth, and factors that affect cyanotoxin production. Complex and synergistic factors, rather than a single dominant parameter, are responsible for cyanobacteria proliferation, and they are typically specific for a given waterbody. HAB case studies specific to Texas waterbodies are also presented.

This literature review cites references from the recent comprehensive work by Chorus and Welker (2021). References also include relevant cyanobacteria and cyanotoxin citations identified in PubMed, Embase, and Google Scholar. Texas case studies were collected from scientific literature, the popular press, and public notifications.

2 Cyanobacteria Properties

2.1 Appearances

Despite the various names that have been used, cyanobacteria are a group of prokaryotic organisms. However, they have historically been considered as algae, and their established nomenclature follows both bacterial and botanical codes, leading to confusion in their taxonomy, which continues even today (Stanier et al., 1978). Cyanobacterial cells can be unicellular or filamentous and can exist singularly or as colonies. Cell sizes range from 0.2 mm to over 40 mm, and their volumes can vary considerably as they form multicellular aggregates. Many species can form mats or colonies by embedding single cells in mucilage, a mucous matrix. They exist as plankton (suspended in the water column or accumulated at surface) or in the benthos (bottom of waterbody). Although cyanobacteria are generally considered photoautotrophic (i.e., they use light and inorganic carbon for energy), they can also behave as heterotrophs (using nutrients and organic carbon from other organisms) and can produce extracellular polysaccharides (EPS), thus forming colonies (Shen et al., 2011; Lea-Smith

et al., 2013). Tables 1-4 summarize some morphological characteristics among common cyanobacteria in their vegetative (active, reproductive) states, and these characteristics are discussed in greater detail below. Appearances can change over their life cycle, in particular when they form akinetes (dormant cells that rest when environmental conditions do not promote growth and germinate when favorable growth conditions return). However, morphology alone may not be sufficient for identifying cyanobacteria. Rather, advanced molecular methods are critical to characterizing cyanobacterial communities (Kurmayer et al., 2004).

2.1.1 Color

Cyanobacteria differ from other bacteria, in that they contain chlorophyll-*a*, similar to plant chloroplasts. They also contain phycobilins, which are accessory pigments bound to water-soluble phycobiliproteins (Tandeau de Marsac 2003). For example, phycocyanin is blue, thus giving cyanobacteria the "blue-green algae" moniker. Phycoerythrin gives some cyanobacteria their red or brown appearance. Carotenoids protect chlorophyll-*a* from oxidative damage and are orange or red. The various ratios of phycocyanin, phycoerythrin, carotenoids, and chlorophyll-*a* can give cyanobacteria various colors ranging from chartreuse to blue-green to violet-red (Vidal et al., 2021).

2.1.2 Bloom formations

Blooms occur in a variety of forms. In general, blooms refer to high phytoplankton cell density that reduces the amount of light passing into a waterbody. Buoyant cyanobacteria, namely *Microcystis*, *Dolichospermum*, and *Aphanizomenon*, that accumulate at or near the surface produce surface blooms in the form of visible streaks and can occur even with low overall cell density. Wind drift at leeward sites can further accumulate cells. Scum can form where buoyant cyanobacteria accumulate at the surface. Surfaces of very dense scums can dry, and cells can lyse, releasing cyanotoxins, as well as pigments and odors. Other cyanobacteria, such as *Oscillatoria*, tend to remain homogeneously distributed in a water column and produce surface scum only under extreme stability. *Planktothrix*, *Aphanizomenon*, *Dolichospermum*, and *Raphidiopsis* cells may also accumulate in deeper layers, such as between the upper warm and deep cold temperatures, or in low illuminance (Ibelings et al., 2021).

2.1.3 Benthic mat formations

Benthic cyanobacteria are common in mats at the bottom of waterbodies. These mats typically contain other organisms, such as heterotrophic bacteria and eukaryotic algae, as well as sediment. EPS hold these components together in mats (McAllister et al., 2016). Mature benthic mats with increased biomass trap oxygen produced from photosynthesis, which results in the mat becoming buoyant. The dynamic shear forces in the waterbody also contribute to mats detaching from the bottom and reaching the surface (Ibelings et al., 2021). Even when waters appear clear and cyanotoxin concentrations in water are low, floating or beached benthic mats pose health hazards for pets and wildlife (Ibelings et al., 2021).

Table 1. Common cyanobacteria morphologies in vegetative states: Toxic planktonic

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Anabaenopsis	Light blue-green	Coiled or straight, 5.3-8.8 µm wide, 5.1-9.3 µm long; may have heterocysts at terminal ends	Free-floating filaments, densely aggregated; contain gas vesicles	Ballot et al. (2008) Baker et al. (2012) Walsby (1972)
Aphanizomenon	Dark brown; filaments may contain pale blue heterocysts	Cylindrical, 5-6 µm in diameter, 8-12 µm long	Unbranched filament, solitary or mat forming; contain gas vesicles	AWWA and WRF (2016) Matthews (2021) Walsby (1972)
Cylindrospermopsis	Blue-green or brown-green	Cylindrical, 1.7-3.0 µm wide, 3-10 µm long	Unbranched filament, straight or coiled; mucilaginous envelope absent; contain gas vesicles	AWWA and WRF (2016) Dordević et al. (2015) Shafik et al. (2003) Saker and Neilan (2001)
Dolichospermum	Light green	Barrel-shaped to spherical, 5.1-11.5 µm wide, 5.0-10.1 µm long; may have round heterocysts	Solitary, straight filaments; contain gas vesicles	Choi et al. (2018)
Microcystis	Protoplast is pale blue-green, but gas vesicles make cells appear dark or brown	Spherical, 2-5 µm	Unicellular and/or irregularly shaped colonies surrounded by mucilage; contain gas vesicles	AWWA and WRF (2016) Connecticut College (2021)
Nodularia (planktonic species most common in brackish waters, some species are benthic in coastal waters without gas vesicles)	Bright blue-green	Discoid or barrel-shaped, 4.0-16 µm wide, 2.0-5.5 µm long; may have spherical, brown, blue or orange heterocysts	Straight or slightly coiled unbranched filaments; may be aggregated into tangled clumps; planktonic species contain gas vesicles	Baker et al. (2012) Matthews (2021) Bolch et al. (1999)
Planktothrix	Green or red	Cylindrical, 3.5-10 µm wide, <4 µm long	Unbranched filament; contain gas vesicles	AWWA and WRF (2016) Baker et al. (2012)

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Pseudanabaena	Green or reddish black	Cylindrical, 0.8-3 µm	Unbranched filament, solitary or agglomerated in very fine, mucilaginous mats; contain gas vesicles	AWWA and WRF (2016) Baker et al. (2012) Acinas et al. (2009)
Raphidiopsis	Pale blue-green, yellowish or olive-green	Cylindrical, 0.8-4.9 µm wide, 4.1-20.5 µm long	Solitary, free-floating filaments tapered toward ends; straight, waved, or helical; contain gas vesicles	Baker et al. (2012) Aguilera et al. (2018)
Sphaerospermopsis	Blue-green	Spherical or slightly elongated, 4-8 µm wide, 4-8 µm long	Solitary, free floating, coiled or straight; contain gas vesicles	Werner et al. (2012)
Synechococcus	Blue-green or red	Cylindrical or rod-shaped, <3 µm wide, <22 µm long	Solitary or in loose colonies; contain gas vesicles	Baker et al. (2012) Damerval et al. (1989) University of Windsor (2021)

Table 2. Common cyanobacteria morphologies in vegetative states: Toxic benthic

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Anabaena (planktonic species now classified as *Dolichospermum*)	Blue-green to yellow-green	Spherical to oblong, 4-14 µm in diameter, 6-12 µm long	Unbranched filament (beaded chain); may form thin, fuzzy mat; may have colorless mucilage	AWWA and WRF (2016) Baker et al. (2012) Ford et al. (2021)
Geitlerinema	Mostly bright blue-green, rarely violet or brown	Oblong, 1.7-2.6 µm wide, 2.6-6.4 µm long	Unbranched, flexuous or straight filament; usually parallel in membrana-ceous mats	Tinpranee et al. (2018) Baker et al. (2012) Komárek et al. (2003)
Hydrocoleum	Olive, pale green, red, pink, violet, orange, yellow, or brown	Cylindrical, 3.0-29.1 µm wide, 0.64-8.2 µm long	Interwoven filaments form firm or loose mats; mats may be flat, compact cushions, or upright tufts	Palinska et al. (2015)

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Lyngbya (some strains have gas vesicles and can be planktonic as well)	Red, brown, black, yellow-brown, slightly red, blue, or gray	Cylindrical, 12.8-36.1 µm wide, 2.4-10.3 µm long	Unbranched, straight filaments; mats resemble fine, silky hair or are clumps; may form thick, floating balls attached by narrow strands to bottom substrate	Sharp et al. (2009) Connecticut College (2021) Engene et al. (2018) Ford et al. (2021)
Merismopedia (some may be planktonic as well)	Pale or bright blue-green, rarely reddish	Spherical to oval, 1.2-6.5 µm in diameter	Cells arranged in rows forming flat and rectangular colonies in mucilage	Baker et al. (2012) Komárek et al. (2003)
Microcoleus (in streams)	Bright, dark or grayish green; occasionally brown, yellow or red	Cylindrical, 3-8 µm wide, 2-6 µm long	Colonies or filaments form thin, compact mats or rope/band-like structures	Strunecký et al. (2013) Baker et al. (2012)
Nostoc (in lakes, on tree trunks and in soggy soil)	Yellow, brown, black, cyan, or emerald	Square, barrel-shaped, spherical, or cylin-drical, 2.0-5.7 µm wide, 2.8-5.7 µm long	Slightly curved filaments form colonies enveloped by gelatinous sheath; colonies can become buoyant, even without gas vesicles	Baker et al. (2012) Singh et al. (2020)
Oscillatoria (planktonic species may be considered *Planktothrix*)	Black, blue-green, green, brown, gray, or purple	Discoid, 1.8-2.4 µm wide, 2.4-4.2 µm long	Filaments form leathery or fuzzy mats	Heath et al. (2010) Ford et al. (2021) Stal et al. (1985) Mühlsteinová et al. (2018)
Phormidium (in streams)	Black, green, brown, or red	Discoid, 3.6-13.2 µm wide, 1.8-6.6 µm long	Filaments form leathery mats	Heath et al. (2010) McAllister et al. (2016)
Scytonema (can also be free-floating)	Yellow-brown, green, blue-green, or gray	Cylindrical or barrel-shaped, 5-18 µm wide, 2.5-10 µm long	Filaments slightly flexuous, often coiled and form tangled mats; may be widened toward ends; often form false branches	Baker et al. (2012) Komárek et al (2013)

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Tychonema (tychoplanktonic; in lakes)	Green or pink	Cylindrical, isodiametric, 4.5-7 µm	Unbranched filaments mainly solitary, straight, or coiled	Baker et al. (2012) Matthews (2021) Shams et al. (2015)

Table 3. Common cyanobacteria morphologies in vegetative states: Toxic but do not form blooms

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Hapalosiphon	Green or brown	Barrel-shaped, 4.8-5.9 µm wide, 5.9-8.5 µm long	Uniseriate trichomes in coiled clusters; initially grow on substrates, but later float among other vegetation	Baker et al. (2012) Nguyen et al. (2017)
Umezakia	Green	Cylindrical, spherical or ellipsoidal, 3-9 µm wide, 4-10 µm long	Filaments may be solitary, free floating, straight, or slightly curved, sometimes with true branches, with thick mucilaginous sheath; may contain gas vesicles	Niiyama et al. (2011)

Table 4. Common cyanobacteria morphologies in vegetative states: Forms blooms with novel toxins

Cyanobacteria	Color	Shape and Cell Size	Cell Aggregation	References
Limnothrix	Black, green, brown, or pale blue-green	Discoid, 1.3-2.4 µm wide, 2.4-10.6 µm long	Filaments may be solitary or entangled to form leathery mats; may contain gas vesicles	Heath et al. (2010) Gkelis et al. (2005)
Gomphosphaeria	Blue-green	Cells club-shaped or heart-shaped, colonies 25-110 µm in diameter	Spherical colonies joined in the middle by mucilaginous stalks that widen at periphery to enclose cells	Baker et al. (2012) Komárek and Komárková-Legnerová (1992)

2.2 Geographic Occurrence

Cyanobacteria can thrive in a wide variety of habitats and adapt to extreme environmental conditions. Thus, they are found around the globe and are not restricted to a specific geography (Pridmore and Etheredge 1987), although specific environments select for specific species (Martiny et al., 2006). While both toxic and nontoxic genotypes of a given species often coexist in both planktonic and benthic mats, the factors that promote nontoxic vs. toxic strains are still under study (Cadel-Six et al., 2007).

2.2.1 Trophic state

Cyanobacteria occurrence is typically associated with the trophic state of a waterbody. Understanding trophic states is important to cyanobacteria management as an indicator of water quality. Although trophic state definitions can be subjective, they are typically related to the amount of biomass produced. Oligotrophic waterbodies produce little phytoplankton biomass, while eutrophic waterbodies produce much more phytoplankton biomass. The delineation between an anoxic hypolimnion (bottom of a thermally stratified waterbody) and oxygenated waters may further define the difference between mesotrophic and eutrophic waterbodies (Carlson and Simpson 1996; Dodds 2006). Benthic cyanobacteria can occur in oligotrophic waters, whereas planktonic varieties do not. Cyanobacterial blooms are rare in mesotrophic waterbodies, except for *Planktothrix rubescens* and detached benthic mats. Cyanobacteria are abundant in eutrophic and hypereutrophic waters (Ibelings et al., 2021).

Trophic states depend on the concentrations of nutrients, especially phosphorus (see discussion on Nutrients below), chlorophyll-*a* as a measure of phytoplankton biomass, and water transparency. Table 5 lists commonly referenced features of trophic states, with total phosphorus as the primary limiting nutrient, as well as associated chlorophyll-*a* concentrations and water transparency as measured from Secchi disc readings (Vollenweider and Kerekes 1982). However, trophic state definitions can be refined further at the local level.

Table 5. Trophic state definitions (adapted from Ibelings et al., 2021)

Trophic State	Total Phosphorus (mean) µg/L	Chlorophyll-a (mean) µg/L	Chlorophyll-a (maximum) µg/L	Transparency (mean) m	Transparency (maximum) m
Ultraoligotrophic	≤4	≤1	≤2.5	≥6	≥12
Oligotrophic	≤10	≤2.5	≤8	≥3	≥6
Mesotrophic	10–35	2.5–8	8–25	3–1.5	6–3
Eutrophic	35–100	8–25	25–75	1.5–0.7	3–1.5

Trophic State	Total Phosphorus (mean) µg/L	Chlorophyll-a (mean) µg/L	Chlorophyll-a (maximum) µg/L	Transparency (mean) m	Transparency (maximum) m
Hypereutrophic	≥100	≥25	≥75	≤0.7	≤1.5

2.2.2 Planktonic cyanobacteria

Globally, the most abundant cyanobacterium is arguably *Prochlorococcus*, which is common in oligotrophic tropical and subtropical oceans, followed by marine *Synechococcus* and *Trichodesium*. Because freshwater accounts for only a small fraction of global surface water, the percentage of planktonic freshwater cyanobacteria is small relative to all cyanobacteria (Garcia-Pichel et al., 2003). Among the planktonic freshwater cyanobacteria are *Microcystis*, which are found worldwide, often in temperatures above 15°C, under mesotrophic to eutrophic conditions, in thermally stratified lakes (deeper than 6 m), and in more shallow waterbodies (Ibelings et al., 1991). *Planktothrix agardhii* is common in shallow, temperate, eutrophic, and hypereutrophic lakes in a wide range of temperatures (Suda et al., 2002; Rücker et al., 1997). However, *Planktothrix rubescens*, which has a red pigment, is more restricted to the metalimnion (zone of rapid temperature change between the hypolimnion and epilimnion in a thermally stratified waterbody) where warm surface waters meet cold deep layers and are thus sensitive to eutrophication and turbidity, which restricts light (Nürnberg et al., 2003). Larger mesotrophic lakes may contain nitrogen-fixing *Dolichospermum* and *Aphanizomenon* with surface warming, high light, and low turbidity (to produce energy for fixing nitrogen) (Reynolds et al., 2002; Porat et al., 2001). Although *Raphidiopsis* is considered a tropical cyanobacteria, it has been found in a wide range of temperatures (20–35°C) (Briand et al., 2004). *Nodularia spumigena* tends to occur in marine and brackish waters, such as estuaries and coastal lagoons (Jones et al., 1994), but a freshwater strain that produced a benthic mat has also been reported (Beattie et al., 2000).

2.2.3 Benthic cyanobacteria

Oligotrophic freshwater and marine environments that allow light to penetrate to the bottom can support benthic cyanobacteria. These varieties attach to sediment (epipsammic), stones (epilithic), or macrophytes (epiphytic). Tychoplanktonic cyanobacteria also typically occur in benthic zones but can thrive in planktonic zones when their benthic habitats are disturbed (Scott and Marcarelli 2012). Benthic *Moorea* (formerly *Lyngbya*) can be found in shallow marine environments in tropical and subtropical zones (Ibelings et al., 2021). Oscillatoriales (*Oscillatoria*, *Planktothrix, Microcoleus*, *Phormidium*, *Microseira*, *Moorea*, *Leptolyngbya*, *Tychonema*, *Calothis,* and *Schizothrix*) and Chroococcales (*Aphanothece* and *Synechococcus*) dominate freshwater benthic mats. Nitrogen-fixing Nostocales (*Anabaena*, *Scytonema* and *Nostoc*) are also common in benthic mats (Quiblier et al., 2013, Wood et al., 2020). When planktonic

cyanobacteria are no longer supported in waters that become clearer, benthic and tychoplanktonic cyanobacteria may replace them (Ibelings et al., 2021).

3 Environmental Factors that Affect Cyanobacteria Growth

Several environmental factors influence when and where planktonic cyanobacteria are likely to grow. Table 6 summarizes the most typical factors, which are discussed in more detail below. However, there are many exceptions to these general observations, and this table should not be used as a guide for all waterbodies. Although temperature is often an important factor, cyanobacteria proliferation varies widely with temperature range; the range is waterbody specific and, therefore, is not included in the table. In general, most or all of the listed conditions, rather than a single condition, are associated with the indicated relative cyanobacterial biomass amount. Note that this table does not include cyanobacteria mats attached to surfaces, such as benthic cyanobacterial mats. Table 7 summarizes common hypotheses to explain cyanobacteria success in freshwater systems (adapted from Mioni et al., 2012).

Table 6. Typical generalized waterbody conditions for high cyanobacterial biomass (Adapted from Burch et al., 2021).

Relative Cyanobacterial Biomass Amount	Total Phosphorus (TP) Concentration	Mixing Conditions and Waterbody Type		Transparency	pH
	>50 mg/L	Stagnant, depth >5-10 m, stable thermal gradients; favors scum-forming taxa (*Microcystis*, *Dolichospermum* and *Aphanizomenon*)	Stagnant, shallow, and well-mixed; favors non-scum-forming taxa (*Planktothrix agardhii* and other fine filamentous forms such as *Limnothrix*)	Low, Secchi depth often <1 m	pH >7 (often >8 or even >9 with high photo-synthesis rates from high biomass)
	20–50 mg/L	Stagnant, deeper than 10 m, stratified; favors *Planktothrix rubescens* at metalimnion		Moderate, Secchi depth ~1–3 m	pH ≥7
	10–20 mg/L	Fast-flowing river	Lake or reservoir with residence time <1 month	High, Secchi depth ~3–7 m	pH 6-7
	≤10 mg/L	Mountain stream or brook		Very high – clear water, Secchi depth often >7 m	pH <6

Table 7. Hypotheses for cyanobacterial success in freshwater systems (adapted from Mioni et al., 2012)

Hypothesis	Advantages for Cyanobacteria	References
Nitrogen speciation	- Some cyanobacteria can fix nitrogen from the atmosphere - Nitrogen from ammonium favors non-nitrogen-fixing cyanobacteria - Urea may be more energetically favorable for cyanobacteria - Other non-nitrogen-fixing cyanobacteria can take up nitrate and nitrite	Schindler et al. (2008) Davis et al. (2010) Gobler et al. (2016) Oliver and Ganf (2000)
Phosphorus reserves	- Cyanobacteria can increase pH, consuming oxygen as cyanobacteria degrade and release phosphorus bound to sediment - Some species in benthic mats release phosphorus from sediments, as well - Cyanobacteria can store excess phosphorus internally	Xie and Xie (2002) Reynolds (2006) Wood et al. (2015)
Low nitrogen to phosphorus ratio	- Ratios may be less of a factor than whether nitrogen or phosphorus is rate limiting - Cyanobacterial populations can shift to other species favored by available nutrients	Reynolds (1999a,b) Chorus and Zessner (2021)
Low light	- Some cyanobacteria have lower light requirements than other phytoplankton - Multiple pigments harvest light in the 500- to 650-nm spectrum range	Reynolds et al. (1981) Reynolds (1997) Wiedner et al. (2007)
Buoyancy	- Many cyanobacteria have gas vesicles that make them buoyant to remain in euphotic zone	Medrano et al. (2013) Humphries and Lyne (1988)
Temperature	- Some cyanobacteria thrive at elevated temperatures, while others prefer moderate temperatures - Elevated temperatures also drive thermal stratification and mixing depth; higher temperatures promote nutrient release, as well	Paerl and Huisman (2008) Srivastava et al. (2013) Walls et al. (2018) Carey et al. (2012) Ibelings et al. (2021)
Hydrodyamics and long residence times	- Buoyant cyanobacteria thrive in thermally stratified waterbodies, whereas large, shallow, turbulent waterbodies favor species that compete well for light - Long residence times promote cyanobacteria that grow slowly	Burch et al. (2021) Ibelings et al. (2021)

Hypothesis	Advantages for Cyanobacteria	References
High pH	- Cyanobacteria can raise pH during photosynthesis - They have a variety of systems for carbonate uptake and can adapt to environments with varying inorganic carbon availability - Some cyanobacteria grow more successfully at high pH	Lampert and Sommer (2007) Chorus and Niesel (2011) Huisman et al. (2018) Sandrini et al. (2014) Becker (1994)
Salinity	- Some cyanobacteria are resistant to osmotic shock	Kruk et al. (2017) Moisander et al. (2002)
Biological agents and grazing	- Blooms can provide refuge for small fish that consume zooplankton, thereby reducing grazing pressure - Many cyanobacteria are resistant to grazing by zooplankton - Zebra mussels selectively reject feeding on cyanobacteria, giving the cyanobacteria a competitive advantage	Engström-Öst et al. (2009) Kurmayer and Juttner (1999) Raikow et al. (2004) Vanderploeg et al. (2001) Dionisio Pires et al. (2005)
Xenobiotics	- Calcium can trigger anti-oxidation and EPS secretion, increasing cyanotoxin buoyancy - Ionic and complexed trace metals can induce cyanobacterial growth	Gu et al. (2020) Zhou et al. (2019)
Overwintering	- Some cyanobacteria can bloom in cold temperatures and keep waters turbid - Other species produce dormant akinetes that sink to sediment until being recruited for germination when warm temperatures return	Ibelings et al. (2007) Takamura et al. (1984) Ihle et al. (2005) Karlsson-Elfgren et al. (2004)

3.1 Nutrients

Cyanobacteria, along with other phytoplankton biomass, consist primarily of carbon, oxygen, hydrogen, nitrogen, and phosphorus. Oxygen and hydrogen are unlimited in water, and carbon concentrations are also not typically limiting. Nutrients enter a waterbody from its catchment, also known as a watershed, river basin, and drainage area. Catchments include point sources, such as discharges and outfalls, and nonpoint sources, such as surface runoff and groundwater inflow. Tile drainage can be an important nutrient contributor to waterbodies, and small lakes and ponds are particularly susceptible to nutrient deposition effects (Mrdjen et al., 2018a).

3.1.1 Nutrient usage, competitive advantage

Cyanobacteria can be found in all environments where water and nutrients are available, even if in small amounts. However, they compete with each other and phytoplankton for nutrients, especially phosphorus and nitrogen. They have several advantages that favor their proliferation. For example, they store excess phosphorus as polyphosphates, which can be used for several cell divisions when external phosphorus concentrations are low (Reynolds, 2006). They can also acquire various forms of nitrogen, such as nitrate, nitrite, ammonium, and urea, and some can even fix atmospheric nitrogen (Gobler et al., 2016; Oliver and Ganf, 2000). Cyanobacteria can also store surplus nitrogen as cyanophycin, meaning that even those species that cannot fix atmospheric nitrogen may have a competitive advantage over other algae in environments where nitrogen concentrations are low (Li et al., 2001).

These organisms also concentrate carbon, which allows them to efficiently use the ribulose bisphosphate carboxylase/oxygenase (RuBisCo) photosynthesis enzyme to fix carbon dioxide (CO_2). The inorganic carbon in the form of bicarbonate is transported to cellular compartments, where carbonic anhydrase transforms the bicarbonate to CO_2, which the RuBisCo can use. Cyanobacteria varieties have different combinations of the carbon uptake systems, which allow them to adapt to environments with varying inorganic carbon availability (Sandrini et al., 2014).

Diverse microbial communities in benthic mats interact when cycling nutrients within the mat (Bouma-Gregson et al., 2019). These mats also promote phosphorus release from sediment within the mats, which is then available for biomass growth (Wood et al., 2015).

3.1.2 Nitrogen

There has been some debate over whether nitrogen or phosphorus is rate limiting for biomass growth. Although adding both nitrogen and phosphorus can increase biomass (Elser et al., 2007; Chorus and Spijkerman, 2021), there is growing evidence that phosphorus is typically more rate limiting, primarily because nitrogen-fixing organisms can utilize inorganic nitrogen from the air (Schindler et al., 2008). Some scholars have proposed lowering waterbody nitrogen concentrations to shift cyanobacterial community composition to nitrogen-fixing species, but such a strategy may not be effective in turbid waters where light availability is limited because nitrogen fixation requires high light energy (Kolzau et al., 2014). Cyanobacteria can also take up dissolved nitrate, nitrite, and ammonium, and some can reduce nitrate to atmospheric nitrogen via denitrification under anoxic conditions (Ibelings et al., 2021). Nitrogen from urea can preferentially stimulate *Microcystis* growth as an energetically favorable nutrient source (Davis et al., 2010), although dissolved inorganic nitrogen concentrations greater than 30–100 µg/L make it unlikely for nitrogen to be the rate-limiting nutrient (Kolzau et al., 2014).

Nitrogen can also enter a waterbody when leached from fertilized soil by runoff and can remain in a waterbody when organic matter decays. Excess nitrogen from fertilizer or animal manure does not tend to bind with soil but is readily soluble as nitrate (nitrogen in urea transforms to ammonium and then to nitrate by nitrification in soil) and leaches from soils to nearby waterbodies (Novotny, 2003). However, nitrogen can limit phytoplankton growth in some eutrophic shallow waterbodies, especially during late summer (Søndergaard et al., 2017). It is important to note that, when nitrogen is the rate-limiting nutrient, reducing the phosphorus concentration can make phosphorus rate-limiting nutrient instead (Ibelings et al., 2021).

3.1.3 Phosphorus

Wastewater, manure fertilizer, and runoff from contaminated soils can provide waterbodies with phosphorus. Because phosphorus is more effective than nitrate in binding with soil particles, surface soil runoff and erosion are the principal mechanisms of entry to waterbodies (Ibelings et al., 2021), although point sources such as wastewater discharge can be important as well and easier to control. Sediment is a sink for phosphorus, but it can also be a source as it cycles between water and sediment, especially in shallow waterbodies with low water exchange rates (Conley et al., 2009; Shatwell and Köhler, 2019). Phosphorus, in the form of apatite with soil, settles to sediment where it remains over long periods of time. Phosphorus may also be bound to iron salts, which dissolve in sediment anaerobic zones, and it can then adsorb to iron- and aluminum oxides and minerals in the sediment. During summer stratification, anoxic conditions can release iron-bound phosphorus from the sediment. In fact, a feedback loop can occur in which cyanobacterial growth raises pH via photosynthesis, which consumes oxygen as it degrades and leads to additional phosphorus release from the sediment (Xie and Xie, 2002). However, phosphorus bound to organic matter is also readily available as organic matter decays (Psenner et al., 1988).

Sufficiently reducing external phosphorus loads to waterbodies has been shown to successfully control cyanobacteria growth in many locations (Phillips et al., 2008; Evans et al., 2011; Carvalho et al., 2013; Søndergaard et al., 2017), but complex biological and chemical phosphorus cycles within waterbodies can make phosphorus available to cyanobacteria years after external loads are reduced (Chorus and Zessner, 2021). Phosphorus interactions with soil also occur on land and can affect surface waters with runoff (Fox et al., 2016). Many agricultural practices include intensive fertilization so that soils can store phosphorus, but soluble phosphorus leaches to nearby waterbodies when it exceeds the soil binding capacity (Behrendt et al., 2000). However, some studies have reported that reducing only phosphorus in coastal waters and estuaries (e.g., Baltic Sea, Wadden Sea, and Gulf of Mexico) has led to excessive nitrogen levels, which may promote harmful algal blooms (Conley et al., 2009).

An understanding of proper phosphorus nomenclature is important when discussing this critical nutrient. Cyanobacteria take up soluble reactive phosphate (SRP), also known as dissolved inorganic phosphate (DIP) or orthophosphate. However, cyanobacteria quickly

take up phosphate released from degrading organic matter, and cyanobacteria can store phosphate for up to four cell divisions, even if no SRP is detected. Thus, SRP measurements are often a poor indicator of the amount of phosphorus relevant to cyanobacterial proliferation. Rather, total phosphate phosphorus drives the amount of phosphorus in cells and should be investigated when managing biomass. Although total phosphorus (TP) includes mineral forms that are not taken up by organisms, TP often represents total phosphate phosphorus. It is also important to note that TP concentrations are reported as the phosphorus atom (30.97 g/mol), rather than phosphate (PO_4, 94.97 g/mol), and mass conversions must account for this (Ibelings et al., 2021).

TP concentrations below 10 µg/L are usually too low for cyanobacteria levels to create health concerns. TP concentrations in the range of 20–100 µg/L drive the amount of cyanobacteria biomass, and biomass levels rarely increase at higher TP concentrations. In fact, higher TP concentrations may drive other resources, such as light, to be rate limiting. (Ibelings et al., 2021). However, the type of waterbody also affects the rate-limiting TP concentration. Some shallow lakes with extensive macrophyte (aquatic plants) cover may not develop cyanobacterial blooms, even when TP concentrations are 100 mg/L (Jeppesen et al., 1991), while large, deep waterbodies can have scums at TP concentrations of 20 mg/L, or *Planktothrix rubescens* in the metalimnion at even lower TP concentrations of 10 mg/L (Chorus and Zessner, 2021).

3.1.4 Nitrogen-to-phosphorus ratios

Some studies have proposed that cyanobacteria can proliferate when the nitrogen-to-phosphorus ratio falls below 29 to 1 (Bulgakov and Levich 1999; Harris et al., 2014), especially in the ratio range of 10 to 15 (Mur et al., 1999). Pawlik-Skowrónska et al. (2013) observed that increasing the nitrogen-to-phosphorus ratio reduced the amount of cyanobacterial biomass but increased the cyanobacterial community variety. However, other studies reported conflicting results in which nitrogen-to-phosphorus ratios were much weaker predictors of cyanobacterial growth than individual nitrogen or phosphorus concentrations (Downing 2001). These contradictory findings can be explained as either or both nitrogen and phosphorus concentrations possibly being too high to be rate limiting, making their ratios irrelevant (Reynolds 1999a,b).

Despite the tendency for phosphorus to be more rate limiting than nitrogen, there are instances in which nitrogen is the rate-limiting factor. Thus, waterbody managers should consider site-specific conditions when assessing the roles of nutrients (Chorus and Zessner, 2021):

1. Shallow, well-mixed waterbodies with short-lived (a few days at most) thermal stratification can drive phosphorus to cycle between water and sediment, making nitrogen a better candidate for controlling biomass growth.

2. Phosphorus may not be rate limiting when phosphorus concentrations are high (i.e., greater than 25–50 mg/L), soluble phosphorus from phytoplankton is elevated (i.e., greater than 5–10 mg/L), or phosphorus is released from the sediment during the summer, when cyanobacteria proliferate.
3. Nitrogen concentrations might be targeted for controlling cyanobacteria growth if they can be reduced to 200–500 mg/L total nitrogen (TN) and to less than 100 mg/L dissolved nitrogen.

It is important to note that limiting nitrogen input can shift phytoplankton communities to nitrogen-fixing varieties, and controlling nitrogen is not an alternative to controlling phosphorus. In fact, rate-limiting nitrogen concentrations are seven to ten times greater than for phosphorus. Rather, nitrogen limitation is an additional approach for overall cyanobacteria management, particularly during summer conditions (Chorus and Zessner, 2021).

3.2 Light

Because cyanobacteria are photoautotrophs, light is a key resource for their growth. When nitrogen and phosphorus are available in excess, greater biomass is produced, making waters turbid. Seasonal changes, such as during darker winter months, can also drive rate limitations toward light when it is less abundant (Ibelings et al., 2021). *Planktothrix agardhii* and *Planktothrix rubescens* can outcompete other organisms in lower light intensities. Other species, such as *Dolichospermum* and those from the Nostocales order, require greater light irradiation (Wiedner et al., 2007).

3.2.1 Buoyancy, competitive advantage

Many species, especially colonial planktonic cyanobacteria, have gas vesicles that give them buoyancy. These gas vesicles tend to develop under low irradiance. Furthermore, when exposed to light, cyanobacteria produce carbohydrates, which can be stored as glycogen. The cyanobacteria, in turn, use glycogen as a ballast and consume it as an energy source in darker depths to regain buoyancy (Medrano et al., 2013). These features allow them to avoid sinking to the sediment where there is low or no light (Ibelings et al., 2021). Eutrophic waterbodies are turbid and limit light penetration to the upper euphotic zone. If water mixing is deeper than the euphotic zone where light is available, phytoplankton's access to light for photosynthesis is partially restricted when they move deeper than the euphotic zone. However, when the water is calm and mixing stops, thermal stratification layers develop, where nonbuoyant plankton sink out of the euphotic zone and buoyant cyanobacteria rise to the surface where light is available (Humphries and Lyne, 1988). *Microcystis* has a particularly large vertical migration span, making it well suited for deep and intermediate eutrophic lakes (Dokulil and Teubner, 2000). Buoyancy regulation can also result in different cyanobacterial accumulation patterns and, thus, cyanotoxin occurrence (Ibelings et al., 2021). As cyanobacteria with tolerance for low illumination intensities bloom, they create a positive feedback loop as

they prevent light from reaching competing organisms, allowing them to dominate (Burch et al., 2021).

3.2.2 Light usage, competitive advantage

Cyanobacteria contain other photosynthetic pigments in addition to chlorophyll-*a*, namely phycocyanin and phycoerythrin. These other pigments harvest light in the 500- to 650¬nm range (green, yellow, and orange parts) of the solar spectrum, which other phytoplankton do not typically use. Thus, they efficiently harvest light and can grow in even low illumination. *Planktothrix*, a more filamentous variety, is a particularly good competitor for light. However, although *Microcystis* has buoyancy advantages, it forms colonies with reduced surface-to-volume ratios, which lowers its overall growth rate (Reynolds, 1997).

3.3 Temperature

Although there are exceptions, the temperature of a waterbody generally affects cyanobacteria growth by:

1. Driving the thermal stratification and mixing depth
2. Increasing the cyanobacteria growth with elevated temperature, as with other organisms (Visser et al., 2016)
3. Increasing organic matter degradation with elevated temperature, and releasing nutrients (Ibelings et al., 2021).

Higher temperatures (25°C or above) have been linked to cyanobacteria growth (Paerl and Huisman, 2008), although temperature alone may not necessarily be a good predictor of blooms (Huber et al., 2012). For example, there are cyanobacteria that can bloom in cooler temperatures. Rather, indirect temperature effects can be more important than direct effects (Carey et al., 2012). Waterbodies in temperate climates thermally stratify in the spring, with increased solar intensity and temperature. Thermal stratification determines the mixing depth in which phytoplankton occurs, the temperatures and nutrient concentrations in the upper mixing zone, whether phytoplankton sinks, whether blooms will accumulate at the surface or remain in suspension, and whether nutrients from decaying biomass remain in the sediment or are mixed in the water column (Ibelings et al., 2021). For example, buoyant bloom-forming cyanobacteria, namely *Microcystis*, are unlikely to proliferate in sustained, well-mixed waterbodies (Visser et al., 2016).

Seasonal changes can also shift cyanobacteria community compositions. Wu et al. (2014) reported that *Microcystis* dominated total phytoplankton biomass from May to December in a large, shallow, eutrophic lake in China. After July, toxic *Microcystis viridis* and nontoxic *Microcystis wesenbergii* dominated, and *Microcystis viridis* became even more pronounced from November to January. Water temperature and nutrient

concentrations were primarily responsible for *Microcystis wesenbergii* growth, whereas nitrite and nitrate concentrations were most associated with *Microcystis viridis* occurrence, although *Microcystis viridis* has been associated with cooler winter temperatures (Takamura and Watanabe, 1987). Another study reported that *Microcystis aeruginosa* was dominant at higher temperatures (24.7–33.9°C), and *Microcystis wesenbergii* was prominent at lower temperatures (19.6–28.6°C) (Imai et al., 2009).

Temperature variations can also affect eutrophic waterbodies dominated by *Planktothrix agardhii.* Walls et al. (2018) found that cyanobacterial biomass in such a waterbody in Ohio increased with warming from 3 to 18°C but decreased at warmer temperatures (20–25°C). Declining biomass led to increased extracellular microcystin (MC) concentrations. However, toxin concentrations do not necessarily increase linearly with cyanobacterial mass, but rather can be at a maximum when environmental conditions for cyanobacterial growth are not optimal (Srivastava et al., 2013; Walls et al., 2018).

Elevated temperatures can also increase phosphorus release and solubility, as well as increasing the activity of aquatic animals that resuspend phosphorus from sediment. This makes temperature a critical factor in cyanobacteria blooms in artificial freshwater aquaculture ponds (Hu et al., 2018).

3.4 Waterbody Size, Shape, and Hydrodynamics

Stratification also depends on the size, depth, shape, wind exposure, and water exchange rate of a waterbody. Buoyant cyanobacteria do not compete well in large, shallow lakes with greater turbulence, especially in the presence of wind. Instead, species such as *Planktothrix agardhii*, which are not strongly buoyant but compete well for light, likely thrive under such conditions. Small, deep, thermally stratified lakes, particularly those that are sheltered from wind, have minimal turbulence and mixing only in the epilimnion (Ibelings et al., 2021). Large, stratified lakes can develop seiches, which are temporary standing wave disturbances that can displace species in the metalimnion, such as *Planktothrix rubescens*. Stable stratification in deep waterbodies also favors phosphorus released from the sediment to remain near the bottom, whereas greater mixing throughout the water column resuspends sediment and its phosphorus (Ibelings et al., 2021).

Waterbodies with regular, well-defined shapes often result in homogeneous water quality and plankton composition. Other waterbodies with irregular shorelines, bays, and tributaries that separate basins create zones in which water quality and bloom formations can vary (Ibelings et al., 2021).

Water residence time, which is estimated from the inflow, outflow, and waterbody volume, can also be an important factor in cyanobacteria proliferation. Cyanobacteria generally grow slowly and require sufficient residence times, often in the range of

weeks, to establish in large numbers (Burch et al., 2021). Low residence times, typically on the order of days, can dilute cyanobacteria sufficiently to lower their ability to multiply (Romo et al., 2013). In temperate climates, cyanobacteria with low growth rates will not outcompete other algal phytoplankton until late summer, although exceptions include *Limnothrix* and *Planktothrix agardhii*, which have high biomasses in summer (Burch et al., 2021). Residence times also affect nutrient concentrations. One study reported that a dilution rate of 10% per day resulted in a nutrient exponential loss rate of –0.16 per day, which limits most phytoplankton growth rates (Reynolds et al., 2012). Long residence times, on the other hand, mean that phosphorus remains available for recycling in a waterbody, even if external nutrient loads are reduced (Jeppesen et al., 1991).

Rivers with rapid flows do not tend to support planktonic cyanobacteria. These rivers can have increased turbidity from high inorganic particle loads, which limit light availability. Benthic grazing and highly fluctuating conditions can also reduce cyanobacteria growth rates in these waterbodies (Dokulil, 1994; Caraco et al., 2006). However, rivers with long stretches of slow flow have fairly constant hydrophysical conditions, and if there are high nutrient concentrations, cyanobacteria that favor well-mixed shallow waterbodies may emerge, such as *Planktothrix agardhii* and other fine filamentous species (Burch et al., 2021). In addition, lowland rivers with low flows can stratify, which may make them suitable for buoyant species such as *Dolichospermum* (Maier et al., 2004).

3.5 pH and Dissolved Inorganic Carbon

Most dissolved inorganic carbon exists as CO_2 in acidic conditions, as bicarbonate (HCO_3^-) at pH 8, and as carbonate (CO_3^{-2}) at high pH values (Stumm and Morgan, 1996). During increased periods of cyanobacterial photosynthesis when carbon uptake exceeds supply, the equilibrium shifts from CO_2 to HCO_3^- and CO_3^{-2}, increasing the pH (Lampert and Sommer, 2007). Thus, large cyanobacterial blooms can cause high pH in a waterbody. Nevertheless, cyanobacteria have a variety of systems for carbon uptake and can be found in waters with both high and low dissolved carbon dioxide concentrations (Huisman et al., 2018). Through carbon overconsumption, they also are known to remove more dissolved inorganic carbon than they require for biomass growth, because they release organic compounds (Schartau et al., 2007). However, one study suggested that increasing dissolved inorganic carbon may suppress toxic *Microcystis* abundance (Yu et al., 2014).

Cyanobacteria are not typically found in acidic waters (pH below 6) (Chorus and Niesel, 2011). In fact, some cyanobacteria grow more successfully at high pH than other phytoplankton (Becker, 1994). Taub (2021) suggested that, as other phytoplankton also consume dissolved inorganic carbon and increase the pH, cyanobacteria may dominate when conditions favor formation of CO_3^{-2}.

3.6 Salinity

Salinity has been considered an important factor for cyanobacterial growth, because it affects osmotic equilibrium and concentrations of the ions responsible for cell function and growth (Silveira and Odebrecht, 2019). Salinity can affect *Microcystis aeruginosa* cluster composition; large-sized colonies with high amounts of mucilage have been reported to be more resistant to osmotic shock and can recover their vertical position after mixing (Kruk et al., 2017). Moisander et al. (2002) similarly found that *Anabaenopsis*, *Anabaena*, and *Nodularia* were able to acclimate to salt stress, although *Cylindrospermopsis raciborskii* had an upper salinity tolerance of 4 g/L NaCl. Low salinity levels have been associated with heterocystous cyanobacteria, while high salinities, which can reduce ammonia volatilization, can make the high nitrogen content detrimental for heterocystous species (Srivastava et al., 2009). Staal et al. (2003) explained that heterocysts develop a glycolipid envelope in less saline conditions, which gives them a selective advantage over non-heterocystous varieties. Because mucilage formation has been linked to toxic varieties, increasing salinity may favor toxic cell growth, and may also release intracellular toxins with lysis (Kruk et al., 2017). Salinity can also affect akinete production, with increased salinity and low temperatures triggering their formation (Silveira and Odebrecht, 2019).

3.7 Biological Agents

3.7.1 Resistance to grazing

Cyanobacteria are resistant to grazing by zooplankton for several reasons, including toxicity, filament or colony size, and poor nutritional quality (Kurmayer and Juttner, 1999), although the extent of such resistance is unclear. Successful grazing depends on both the zooplankton and cyanobacteria type (Mohamed, 2001; Wilson et al., 2006), and zooplankton can develop a tolerance against cyanotoxins (Ger et al., 2016). Moreover, there is evidence that in *Microcystis* colonies that have been grazed upon by protozoans, the remaining ungrazed cells had greater photosynthetic growth from protozoan nutrient recycling (Paerl and Millie, 1996).

3.7.2 Zebra mussel impacts

Zebra mussels (*Dreissena polymorpha*) typically intensively filter feed plankton biomass. Adults of this invasive species have been observed to selectively reject feeding on toxic *Microcystis* colonies while consuming algae, thus giving cyanobacteria a competitive advantage and promoting growth (Raikow et al., 2004, Vanderploeg et al., 2001). Other research has demonstrated that zebra mussels consume both toxic and nontoxic *Microcystis* at the same rate and excrete them as pseudofeces (Dionisio Pires et al., 2005). However, juvenile zebra mussels can be susceptible to cyanotoxins with suppressed production and development (Boegehold et al., 2019). One conflicting study concluded that such zebra mussel grazing behavior is not consistent, because some locations experienced a decrease in *Microcystis* abundance in the presence of zebra

mussels (Horst et al., 2014; Smith et al., 1998). In addition, zebra mussels consume both phytoplankton and zooplankton, thereby removing *Microcystis* competitors (Harke et al., 2016a).

Zebra mussels can also increase dissolved organic phosphorus concentrations, altering the nutrient profile to favor *Microcystis* (Heath et al., 1995; Harke et al., 2016a). Zebra mussels may act as a sink for phosphorus (Dolan and Chapra, 2012), as well as a source of soluble reactive phosphorus, because they mobilize iron-bound phosphorus from sediments in their anoxic guts (Turner, 2010).

Other waterbody changes attributed to zebra mussels include water chemistry. These mussels have been observed to take up calcium and store it in their shells, thus reducing water alkalinity. This can result in competition among primary producers for dissolved CO_2 for which *Microcystis* has an advantage. Inorganic carbon tends to convert to bicarbonate under increased pH conditions. *Microcystis* can use bicarbonate as carbonic anhydrase can convert it to CO_2 before it enters the cell, making CO_2 even less available for competitors (Aizawa and Miyachi, 1986; Kotak et al., 2000; Poste et al., 2013; Rinta-Kanto et al., 2005).

3.7.3 Predators

Despite their resistance to grazing and their toxicity, some organisms can help to control cyanobacterial growth (Sigee et al., 1999). Certain fish that consume phytoplankton, such as silver carp and bighead carp, can filter feed on cyanobacteria (Zhang et al., 2008). Fungal parasites, cyanophage, heterotrophic bacteria, actinomycetes, and protozoa can contribute to cyanobacteria losses, although these relationships are still being studied (Sigee et al., 1999; Ibelings et al., 2021). Manage (2009) reported that (in descending order) cyanophages, algicidal bacteria, rotifer *Brachionus calicifloroous*, zooplankton *Cephalodella* sp., testate amoeba *Penardochlamys* sp., and protozoa *Polytomella* sp. helped to suppress *Microcystis aeruginosa* growth in a lake in Japan during the normal bloom period. In addition, cyanophage lysis events have resulted in MCs being released from blooms (McKindles et al., 2020). Phage life cycles can be seasonal—one study found lysogeny genes during early (June to July) and late (October) blooms, with lytic genes from late July to October (Stough et al., 2017). However, other studies have reached conflicting conclusions where the phage did not appear to reduce the size of dense blooms (Rozon and Short, 2013), and some *Microcystis* can develop defenses against phage infections (Harke et al., 2016b). Ndlela et al. (2018) reviewed studies using bacteria as cyanobacterial controls and concluded that temperature, pH, and nutrient conditions may lead to reduced performance outside of laboratory-scale studies. Fabbro et al. (2001) reported that *Paramecium* successfully grazed on *Cylindrospermopsis*. Amoeba predation on cyanobacteria is varied, and possibly preferential for benthic cyanobacteria, although thick mats may inhibit grazing (Ma et al., 2016). Furthermore, Wilken et al. (2014) observed that, at low nitrogen concentrations, *Ochromonas*, an algae known to compete with and prey on *Microcystis*,

suppressed *Microcystis* abundance, but lost control over the *Microcystis* population at high nitrogen loads.

Zooplankton are an important link in the food web between primary producers, such as phytoplankton, and higher trophic levels, such as fish. Cyanobacteria effects on zooplankton depend on the species. Increased cyanotoxin production can trigger shifts from large-bodied to small-bodied zooplankton, as well as increased rotifer, copepod, and small-bodied cladoceran biomass, presumably because these organisms can select against toxic cells (Reichwaldt et al., 2013). Other organisms, namely *Daphnia*, starve in the presence of cyanobacteria (Lampert, 1981). In addition, algal blooms, including those from toxic cyanobacteria, can serve as a refuge for small fish against predation, which then consume large zooplankton and reduce grazing pressure (Engström-Öst et al., 2009).

3.8 Xenobiotics

Gu et al. (2020) observed that metals can affect *Microcystis aeruginosa*, with copper (>0.1 mg/L) and lead (>1 mg/L) suppressing the growth rate, but that calcium (>100 mg/L) and cadmium (<0.1 mg/L) facilitated blooms. The high concentrations of calcium triggered anti-oxidation, which promoted EPS secretion, aggregated cells, and significantly increased their buoyancy. Zhou et al. (2019) reported the order of metal ion toxicity to *Microcystis aeruginosa* as copper > zinc > iron. This same study observed that ionic and complexed trace metals induced more cyanobacterial growth than carbonate and sulfide-bound species.

3.9 Overwintering

In warmer climates, cyanobacteria can bloom year-round. For example, *Planktothrix*, among other cyanobacteria, can bloom even during winter months, keeping the water system turbid and preventing light from reaching other algae (Ibelings et al., 2007). Other cyanobacteria have overwintering strategies to remain viable in temperate climates during the colder months. During autumn and winter, the mixing depth of a waterbody deepens, reducing the amount of light available for photosynthesis, which reduces the size of cyanobacteria blooms. During this time of bloom reduction, viable *Microcystis* cells may sink to the sediment and become available to form blooms the following summer (Takamura et al., 1984; Ihle et al., 2005). Nitrogen-fixing *Anabaena*, *Dolichospermum*, *Aphanizomenon*, and *Raphidiopsis* produce akinetes, which are dormant cells that sink and can survive dormant for extended time periods until they germinate under favorable conditions (Karlsson-Elfgren et al., 2004).

4 Factors that Affect Cyanotoxin Concentrations

4.1 Cyanotoxins and Genetics

Cyanobacteria can occur in a wide variety of environments, and therefore, cyanotoxins are also found in most waterbodies. Cyanotoxin concentrations are closely related to the amount of toxic cyanobacteria biomass. Common cyanobacteria and their dominant associated toxins are listed in Tables 8-11 and grouped based on where they are typically found (i.e., planktonic vs. benthic). However, these tables are not exhaustive, and the scientific community's understanding of cyanobacteria taxa and their toxins continues to evolve. Appendix A provides existing regulatory, as well as candidate microcystin-LR (MC-LR), cylindrospermopsin (CYN), and anatoxin-a (ATX) screening levels for humans and animals.

The most common types of cyanotoxins with concentrations relevant to health effects are MCs, which are hepatotoxins and dermatoxins (USEPA, 2015). These are typically found with *Microcystis* and *Planktothrix* (Svirčev et al., 2019). Although *Microcystis* and *Planktothrix* blooms can contain clones both with and without genes for MC production, there are nearly always toxic clones (Kurmayer and Gumpenberger, 2006; Welker et al., 2004). *Oscillatoria*, *Nostoc*, *Anabaena*, *Anabaenopsis*, *Leptolyngbya*, and *Geitlerinema* cyanobacteria also produce MCs (Kaebernick and Neilan, 2001). The *mcyA-J* gene cluster is responsible for MC production in which a multienzyme complex allows MC components to be assembled non-ribosomally (Kaebernick and Neilan, 2001; Dittmann and Börner, 2005).

Nodularins (NODs), another type of hepatotoxin, are associated only with *Nodularia spumigena* (Kaebernick and Neilan, 2001). NOD biosynthesis occurs using a non-ribosomal mechanism similar to that used in MC production (Dittmann and Börner, 2005; Dittmann and Wiegand, 2006).

Several types of cyanobacteria are known to produce cylindrospermopsin (CYN), which are hepatoxins and nephrotoxins. These species include *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Raphidiopsis curvata*, and *Umezakia natans* (Banker et al., 1997; Fristachi and Sinclair, 2008), as well as *Nostocales*, *Stigonematales*, and *Oscillatoriales* (Christensen and Khan, 2020). These cyanobacteria also use genes that gather CYN components non-ribosomally (Schembri et al., 2001).

Table 8. Common cyanobacteria and their cyanotoxins: Toxic planktonic

Cyanobacteria	Microcystin (MC) (>250 variants[b])	Nodularin (NOD) (8 variants[a])	Cylindrospermopsin (CYN) (5 variants[e])	Anatoxin-a (ATX) (5 variants[g,n])	Saxitoxin (STX) (20 variants[a])
Anabaenopsis [a,b,c,d,h,l]	X			X	
Aphanizomenon [a,c,d,e,h,k,l]	X		X	X	X
Cylindrospermopsis [a,c,d,h,j,l]			X	X	X
Dolichospermum [b,k]			X	X	
Microcystis [a,b,c,d,h.j,l]	X			X	
Nodularia (planktonic most common in brackish waters, some are benthic in coastal waters) [a,b,c,d,h,j]		X			
Planktothrix [a,b,c,h,j,k,l,]	X			X	X
Pseudanabaena [b,d]	X				
Raphidiopsis [a,b,c,d,e,h,k]			X	X	X
Sphaerospermopsis [a,k]			X		
Synechococcus [a,b,c,d]	X				

Table 9. Common cyanobacteria and their cyanotoxins: Toxic benthic

Cyanobacteria	Microcystin (MC) (>250 variants[b])	Nodularin (NOD) (8 variants[a])	Cylindrospermopsin (CYN) (5 variants[e])	Anatoxin-a (ATX) (5 variants[g,n])	Saxitoxin (STX) (20 variants[a])
Anabaena (planktonic species now classified as *Dolichospermum*) [a,b,c,d,j,k,l,m,p]	X		X	X	X

Cyanobacteria	Microcystin (MC) (>250 variants[b])	Nodularin (NOD) (8 variants[a])	Cylindrospermopsin (CYN) (5 variants[e])	Anatoxin-a (ATX) (5 variants[g,n])	Saxitoxin (STX) (20 variants[a])
Geitlerinema [i,m]	X			X	X
Hydrocoleum [f]				X	
Lyngbya [a,c,d,j,k,l]			X		X
Merismopedia [b]	X				
Microcoleus (in streams) [g,k,m]				X	
Nostoc (in lakes and on tree trunks and soggy soil) [a,b,c,d,k,l.m]	X	X			
Oscillatoria (planktonic species may be considered *Planktothrix*) [a,b,c,d,e,j,k,m]	X		X	X	X
Phormidium (in streams) [a,b,c,d,h,j,k,m]	X			X	X
Scytonema (can also be free-floating) [a]					X
Tychonema (tychoplanktonic in lakes) [m]				X	

Table 10. Common cyanobacteria and their cyanotoxins: Toxic but do not form blooms

Cyanobacteria	Microcystin (MC) (>250 variants[b])	Nodularin (NOD) (8 variants[a])	Cylindrospermopsin (CYN) (5 variants[e])	Anatoxin-a (ATX) (5 variants[g,n])	Saxitoxin (STX) (20 variants[a])
Hapalosiphon [b,c,d,j]	X				
Umezakia [a,c,d,h,j,l]			X		

Table 11. Common cyanobacteria and their cyanotoxins: Forms blooms with novel toxins

Cyanobacteria	Microcystin (MC) (>250 variants[b])	Nodularin (NOD) (8 variants[a])	Cylindrospermopsin (CYN) (5 variants[e])	Anatoxin-a (ATX) (5 variants[g,n])	Saxitoxin (STX) (20 variants[a])
Limnothrix [n]					
Gomphosphaeria [o]					

References: [a]Boopathi and Ki (2014), [b]Fastner and Humpage (2021), [c]Fristachi and Sinclair (2008), [d]Graham (2021), [e]Humpage and Fastner (2021), [f]Méjean et al. (2010b), [g]Puddick et al. (2021), [h]Řezanka and Dembitsky (2006), [i]Richardson et al. (2007), [j]Sivonen (2009), [k]Testai (2021), [l]Westrick et al. (2010), [m]Wood et al. (2020), [n]Humpage et al. (2012), [o]Berg et al. (1986), [p]Merel et al. (2013).

Anatoxin-a (ATX-a) congeners, including ATX-a, dihydroanatoxin-a (dhATX), homoanatoxin-a, and dihydrohomoanatoxin-a (dhHTX), are neurotoxins mainly associated with *Anabaena*, *Aphanizomenon,* and *Planktothrix* (Osswald et al., 2007; van Apeldoorn et al., 2007). Varieties of ATX-a have also been found widely in benthic mats containing *Anabaena*, *Phormidium* (Bouma-Gregson et al., 2018), *Geitlerinema* (Cantoral Urizea et al., 2017), *Hydrocoleum* (Méjean et al., 2010b), and *Microcoleus* (Puddick et al., 2021). However, anatoxin-a(s) (ATX-a(s)) has been associated only with *Anabaena* strains (Merel et al., 2013). Genes responsible for ATX production have been identified, although the biosynthesis mechanism is not yet well understood (Cadel-Six et al., 2009; Méjean et al., 2010a). Heath et al. (2014) reported that dhATX and dhHTX can account for the majority of ATX production by *Phormidium*. Furthermore, Puddick et al. (2021) observed that dhATX is likely among the most toxic ATX congeners by oral ingestion, raising concerns about its presence because it has been associated with animal deaths around the world.

In freshwater bodies, saxitoxins (STXs), which are also neurotoxic, are produced primarily by *Anabaena circinalis* and *Aphanizomenon flos-aquae*, although *Lyngba wollei*, *Cylindrospermopsis raciborskii*, and *Raphidiopsis bookii* can also produce STXs (Nicholson et al., 2003). Some dinoflagellates can also produce STXs in seawater (Merel et al., 2013).

A recently discovered neurotoxic cyanotoxin, b-N-methylamino-L-alanine (BMAA), can be produced by most known groups of cyanobacteria, especially *Nostoc* (Cox et al., 2005). Diverse cyanobacteria taxa have genes that code for enzymes involved in BMAA biosynthesis (Aráoz et al., 2008). Other marine cyanobacteria neurotoxins have also been reported recently as being produced from *Moorea producens* (formerly *Lyngbya majuscule*)*,* namely antillatoxin, kalkitoxin, and jamaicamide (Aráoz et al., 2010).

Moorea producens is also known to produce dermatoxins such as aplysiatoxins and lyngbyatoxins. However, these have been detected only in marine waters (van Apeldoorn et al., 2007).

4.2 Clonal Composition

Toxic and nontoxic cyanobacteria often coexist in a given population. Only strains that possess the appropriate genes produce cyanotoxins (Kurmayer and Christiansen, 2009), and these strains can turn certain genes on or off according to environmental conditions (Merel et al., 2013). Many researchers have attempted to correlate cyanotoxin content in cells with factors such as nutrients, light, and temperature, but have produced contradictory results; potential correlations remain poorly understood, although there may be multiple complex triggers for cyanotoxin production (Harke et al., 2016a; Ibelings et al., 2021). Additionally, factors that affect overall cyanobacteria biomass proliferation, not just individual toxic clones, may primarily affect cyanotoxin concentrations (Salmaso et al., 2014). In addition, microscopy is not reliable for

identifying cyanobacteria; advanced molecular methods have emerged as the standard in characterizing cyanobacterial communities (Kurmayer et al., 2004).

Although cyanotoxin concentrations typically are correlated to the amount of cyanobacteria available, measured as chlorophyll-*a* or biovolumes of specific taxa, there are upper bounds to these relationships below which there can considerable variability in the ratios of cyanotoxins to cyanobacterial biomass (Ibelings et al., 2021). Composition shifts between clones with different toxin contents can explain this variability (Otten et al., 2017). The amount of cyanotoxin in a given clone can also vary by a factor of two to four (Ibelings et al., 2021). In natural waters, cyanobacterial toxic clonal population dynamics vary over time, thus changing cyanotoxin concentrations (Wood and Puddick, 2017). For example, CYN and neurotoxins have been found in blooms containing mixtures of toxic and non-toxic clones (Fastner et al., 2001; Wood and Puddick, 2017). Kelly et al., (2019) also observed that mats in which cyanobacteria were not the dominant taxa still may have concentrations of cyanotoxins, such as ATX and NOD, similar to concentrations in cyanobacteria-dominated mats.

Seasonal patterns of cyanotoxin concentration correlations to biomass can vary among waterbodies. Some studies have reported that waterbodies with *Planktothrix* and *Microcystis* communities had a maximum MC content when blooms first occurred and declined throughout the season (Kardinaal et al., 2007; Davis et al., 2009), whereas other researchers reported opposite observations (Ibelings et al., 2021). Some waterbodies have been observed with stable proportions of toxic clones to total cell numbers (Salmaso et al., 2014). There may be occasions when ATX producers temporarily dominate waterbodies that contain MC-producing taxa (Bouma-Gregson et al., 2018). Furthermore, maximum MC concentrations can result from a high abundance of toxic cyanobacteria, even if the amount of MC in the cells is low (Ibelings et al., 2021).

4.3 Spatial Variability

Cyanotoxin concentrations are heterogeneous, as are cyanobacterial biomasses. Toxin concentrations can vary by several orders of magnitude, even in the same waterbody on the same date, depending on where and how the sample is collected. Horizontal variability of cyanotoxin concentrations results from the different growth conditions, as well as horizontal biomass dislocation, because cells generally concentrate near shore and in the downwind sites of a waterbody (Chung et al., 2014; Miller et al., 2019). Vertical variability is caused mainly by buoyancy regulation, particularly with buoyant cyanobacteria, such as *Microcystis*, dominating the waterbody surface (Naselli-Flores et al., 2007) and *Planktothrix rubscens* in the metalimnetic layer (Ernst et al., 2009). For example, MC concentrations have frequently been found in the mg/L range in *Microcystis* scums, but in much lower concentrations where cyanobacteria were more evenly distributed in a water column (Cook et al., 2004; Loftin et al., 2016).

4.4 Environmental and Biomass Conditions

Although cyanobacteria biomass, and therefore cyanotoxin, concentrations are highly variable with space and time, there are several factors that can aid in estimating cyanotoxin concentrations. These are, in order of increasing estimate uncertainty:

1. Cyanobacterial biovolumes
2. Cyanobacterial cell counts
3. Chlorophyll-*a* concentrations
4. Water transparency (Secchi depth)
5. Nutrient concentrations.

4.4.1 Cyanobacterial biovolumes

Biovolume measurements offer the most accurate estimate of maximum expected cyanotoxin concentrations in cells. However, variable toxin content among clones makes the estimates reasonable only to within an order of magnitude (Ibelings et al., 2021). Nevertheless, field samples and laboratory studies have suggested that a maximum ratio of 3 mg MC per mm^3 biovolume can generally be expected in waterbodies, although greater ratios have been reported (Hesse and Kohl, 2001; Znachor et al., 2006).

Although the maximum CYN cellular content is in the same range as for MCs, proportions of CYN dissolved in water can be higher than cell-bound CYN and can persist even after the cyanobacteria have been removed. Thus, if cyanobacteria that are known to produce CYN, such as *Raphidiopsis raciborskii* (North America, South America, and Australia) and *Aphanizomenon* (Europe) are present, CYN concentrations should be measured directly (Ibelings et al., 2021).

4.4.2 Cell counts

Toxic cyanobacteria cell counts can be used, along with associated toxin cell quotas (amount toxin per cell, usually expressed as femtogram [fg] per cell), to estimate concentrations of cyanotoxins. However, this approach is most suitable for taxa with available cell quota estimates, and these data are scarce. Just as with biovolumes, toxin content variability also introduces uncertainty using this method (Ibelings et al., 2021). *Microcystis* cell quotas from field samples can range from 1 to 144 fg/cell (Okello et al., 2010). CYN cell quotas for *Raphidiopsis raciborskii* and *Chrysosporum ovalisporum* have been reported as 60 fg/cell (Orr et al., 2011) and 191 fg/cell (Vasas et al., 2013), respectively. A saxitoxin cell quota of 1.3 picogram (pg) per cell has been found in *Scytonema* (Smith et al., 2011). Cyanotoxins that do not have known cell quotas are treated conservatively as MC, because their maximum cellular concentrations are similar to those of MCs, although actual concentrations are substantially lower than MCs (Ibelings et al., 2021).

4.4.3 Chlorophyll-a concentrations

Chlorophyll-*a* concentrations measure total phytoplankton organisms, including cyanobacteria. Thus, qualitative microscopic analysis would be needed to determine the prevalence of cyanobacteria in a sample when using chlorophyll-*a* as a cyanotoxin indicator. Phytoplankton chlorophyll-*a* content varies with light and nutrient availability, which introduces uncertainty to this method. Correcting for phycocyanin, a pigment found only in cyanobacteria, can also help to improve the reliability of this method (Ibelings et al., 2021). Studies have reported that MC concentrations in cells do not normally exceed the chlorophyll-*a* concentration, and the typical ratio is between 0.1 and 0.5 (Sinang et al., 2013; Loftin et al., 2016; Mantzouki et al., 2018.) Therefore, a conservative approach assumes a maximum ratio of 1 mg MC per mg chlorophyll-*a*, provided it is largely from cyanobacteria (Ibelings et al., 2021).

4.4.4 Water transparency

Water transparency is easily measured with a Secchi disk on site and is often correlated with phytoplankton and chlorophyll-*a* concentrations. Subbiah et al. (2019) found that increased turbidity was directly related to increased ATX and MC concentrations. Although suspended inorganic sediments and humic substances can also affect water transparency, high Secchi depths (increased water transparency) generally indicate lower cyanobacteria levels and cyanotoxin concentrations in the epilimnion (upper layer). Transparency thresholds for cyanobacterial blooms depend on the given waterbody or waterbody type, as well as the season (Ibelings et al., 2021). It is important to note that Secchi disk readings may not be useful for indicating benthic or tychoplanktonic cyanobacteria, as well as *Planktothrix rubescens*, in the metalimnion.

4.4.5 Nutrient concentrations

TP and, often to a lesser extent, TN typically determine the maximum amount of phytoplankton biomass. In turn, they can also determine the maximum amount of cyanotoxin concentrations (Dolman et al., 2012). For example, Pawlik-Skowrónska (2013) found that ATX production was positively correlated with total cyanobacterial biomass. However, nutrient concentrations are more useful as long-term factors for cyanobacteria blooms and cyanotoxins (Beaver et al., 2014). Nevertheless, research continues into the role of nutrients in promoting toxigenic variants. For example, MC-producing cyanobacteria are resistant to nutrient limitations (Pimentel and Giani, 2014)—in particular, iron (Sevilla et al., 2008), nitrogen (Horst et al., 2018; Kotak et al., 2000) and phosphorus (Ginn et al., 2010; Pawlik-Skowrónska et al., 2013). This protective mechanism is believed to result from MC binding to proteins under oxidative stress that results from nutrient limitations, increasing the fitness of toxic strains (Pimentel and Giani, 2014). Nitrogen starvation has been similarly linked to ATX production (Neilan et al., 2013). Increased nitrate concentrations can also promote MC production in some non-nitrogen-fixing cyanobacteria (Boopathi and Ki, 2014). Furthermore, addition of nutrient-rich guano from pelicans to a lake in Greece was also demonstrated to increase MC-YR and MC-LR production at the expense of MC-RR,

possibly because of micronutrients not otherwise available (Maliaka et al., 2020). Nutrient concentrations and forms can affect CYN production as well, with CYN production increased from *Cylindrospermopsis raciborskii* under a lack of a fixed-nitrogen source, and phosphate addition may favor toxic CYN-producing strains (Burford et al., 2014). Inorganic nitrogen may also favor nontoxic *Microcystis* strains (Davis et al., 2010).

Nutrient concentrations can also affect relationships between cyanobacterial species. Chia et al. (2018) reported that *Microcystis* strongly suppressed *Anabaena* under high nitrogen and low phosphorus concentrations, whereas the reverse occurred under low nitrogen conditions. When *Microcystis* had the competitive advantage, it produced greater amounts of MC and suppressed ATX production from *Anabaena*, presumably from an allelopathic relationship (where one type of plant inhibits the growth of another). Other studies have also suggested that allelopathic interactions, as non-cyanobacterial phytoplankton competitors, release EPS, which may stimulate MC production (Bittencourt-Oliveira et al., 2014; Sinang et al., 2015).

4.5 Meteorological Conditions

MC congeners can vary with environmental conditions. Continent-scale temperature differences have been suggested as primarily determining cyanotoxin diversity (Mantzouki et al., 2018). Taranu et al. (2019) reported that globally MC-LR dominated, with strong winds, warm temperatures, and nutrient-rich conditions. MC-LA, which was reported more commonly in North America than Europe, was associated with intermediate winds, wetter meteorological conditions, and poorer nutrient availability. Zhou et al. (2016) similarly observed that short-term high winds that produced turbulence in lakes triggered toxic *Microcystis* species growth, and thus increased concentrations of MCs. Another study confirmed that MC-RR and MC-LR dominated in high winds when coupled with high temperatures (Mischke, 2003). The increased turbulence in this study also raised shear stress, which could have lysed cells and released MCs. However, variability exists even within regions—maximum cyanotoxins were reported in late summer for some lakes but in early spring for another lake near Quebec, Canada (Rolland et al., 2005).

Ultraviolet (UV) radiation can affect MC production because nontoxic cyanobacteria can be more vulnerable to UV than toxic variants (Ding et al., 2013). Phelan and Downing (2011) suggested that MCs can protect toxigenic cells against photo oxidation under high light intensity. Light and heat stress can similarly promote NOD (Pearson et al., 2010) and STX production (Boopathi and Ki, 2014).

4.6 Xenobiotics

Lanthanum(III), a rare earth element widely used in electronics and other technology, is an emerging contaminant in many waterbodies. This element enters the environment through consumer and industrial product disposal via landfills, mining and mineral processing discharges, and industrial wastewater effluent (Gwenzi et al., 2018). This co-pollutant can trigger *Microcystis aeruginosa* cells to rapidly absorb nutrients, which in turn stimulate chlorophyll production, photosynthesis, and MC-LW, MC-LR, and MC-YR production (Liu et al., 2020a). Similarly, Wang et al. (2018) demonstrated that iron increases *Microcystis aeruginosa* cell growth, upregulates photosynthetic capacity, and promotes MC-LR production. Titanium dioxide nanoparticles are used in many fields, including foods, gas sensors, photocatalytic media, paint, cosmetics, and personal care products, and are increasingly being released to the environment and waterbodies. At reduced pH (6) or temperature (20°C), titanium dioxide nanoparticles can coat *Microcysts aeruginosa*, which hinders light absorption and cellular growth but may increase MC-LR production (Wu et al., 2019; Zhang et al., 2020). Furthermore, high concentrations of linear alkylbenzene sulfonate, another emerging pollutant that is used widely in industrial and domestic applications, increases MC production from *Microcystis aeruginosa* (Wang et al., 2015). The use of low-dose hydrogen peroxide is an attractive method to mitigate cyanobacterial blooms, but the simultaneous presence of antibiotics may stimulate *Microcystis aeruginosa* growth and MC synthesis (Liu et al., 2020b). However, Gao et al. (2020) reported that toxic *Microcystis aeruginosa* clones were more sensitive to pyrogallol, a plant allelochemical, than nontoxic clones; this is significant because pyrogallol has been proposed as an anti-cyanobacterial agent.

5 Drinking Water Concerns

In general, human health concerns may result from cyanotoxins being released from cyanobacterial cells and entering drinking water sources. Seasonal environmental changes, predation, viral attacks, and algaecides can lyse cells and release their toxins (Westrick et al., 2010). When extracellular cyanotoxins or cyanobacteria containing intracellular cyanotoxins enter the intake for a drinking water system, the water must be treated or the intake adjusted to prevent cells or toxins from entering the treatment facility. Treatment-method efficacy varies among extracellular cyanotoxins, depending on their chemical and physical properties, such as hydrophobicity, hydrophilicity, molecular size, and functional groups (Westrick et al., 2010). Conventional drinking water treatment methods are discussed below in terms of the ability of each to remove intracellular (intact cyanobacteria) and extracellular cyanotoxins. In many cases, an integrated approach using multiple methods is suitable (Antoniou et al., 2014).

5.1 Intake Management

Because cyanobacterial communities and behaviors are waterbody specific, drinking water treatment operators must understand the cyanobacteria patterns when using a surface waterbody as a drinking water source. Treatment-plant intake can be adjusted to draw water from different depths or at different times to avoid introducing cyanobacteria and cyanotoxins into the treatment facility (AWWA and WRF, 2016).

5.2 Cyanobacterial Cell Removal

Cyanobacterial cells are fragile, so treatment processes should consider removing the cells, when possible, before they release their toxins (AWWA and WRF, 2016; de Figueiredo, 2004). Cells can be removed using conventional coagulation, flocculation, and sedimentation; dissolved air filtration; and filtration.

5.2.1 Coagulation, flocculation, sedimentation, and filtration

Conventional coagulation, flocculation, and sedimentation treatment uses chemical coagulants to neutralize cyanobacterial cell negative charges so they can form flocs and settle out. This process, especially when followed by filtration, can be effective in removing intact cells (Gheraout et al., 2010), although it has also been known to induce MC release from cells (Mohamed et al., 2015). The process must be optimized to account for variability among cyanobacterial blooms (Ghernaout et al., 2010). Full-scale treatment facility operational parameters should be set after conducting jar tests to optimize coagulant dose, pH, and settling time (AWWA and WRF, 2016).

5.2.2 Dissolved air flotation

Dissolved air flotation (DAF) introduces bubbles into water, and the bubbles attach to solid particles, which makes them less dense so they float to the water surface. The floated material can be removed by a skimmer (Antoniou et al., 2014). Thus, DAF has been demonstrated as effective for removing cyanobacteria with low densities and high buoyancy (Teixeira and Rosa, 2007). However, care should be taken to not subject cyanobacteria to shearing stresses from the pressurized air, which can damage and lyse the cells (Antoniou et al., 2014). In many cases, DAF combined with coagulation/flocculation can remove cells more efficiently than coagulation, flocculation, and sedimentation alone (Teixeira and Rosa, 2006).

5.2.3 Membrane filtration

Membrane filtration, namely microfiltration and ultrafiltration, effectively removes cyanobacterial cells by size exclusion, even without upstream coagulation (AWWA and WRF, 2016). Nanofiltration and reverse osmosis can remove cyanotoxins as well (AWWA and WRF, 2016; Gijsbertsen-Abrahamse et al., 2006). However, coagulation as a pretreatment can improve membrane filtration by reducing fouling (Huang et al., 2009).

5.3 Oxidants

The drinking water industry commonly uses oxidants, such as chlorine, UV irradiation, potassium permanganate, and ozone, to disinfect and degrade contaminants. These oxidants can be introduced at the intake (pre-coagulant), as a filter aid (pre-filter), or as a disinfectant (post-filter) (AWWA and WRF, 2016). However, if these oxidants also lyse cyanobacterial cells, additional oxidant dosing may be required to account for both lysing and reactions with cyanotoxins. Therefore, treatment plant operators should consider physically removing cells prior to oxidation processes (Onstad et al., 2007).

5.3.1 Chlorination and disinfection byproducts

Chlorine, a common drinking water disinfection agent, has been demonstrated to degrade MC, CYN, STX, and NOD, thus reducing water toxicity (Ho et al., 2008; Liu et al., 2020c; Senogles et al., 2000). ATX-a, on the other hand, appears to resist chlorination (Merel et al., 2010). However, chlorine, namely in the form of hypochlorous acid (HOCl), can react with organic compounds to produce disinfection byproducts (CDC, 2016). Cyanotoxins, which are organic compounds, can thus react with chlorine to form disinfection byproducts such as trihalomethanes, haloacetic acids, haloacetonitriles, and organic halogens (Liu et al., 2020c; Tsuji et al., 1997). Chlorine added at the intake can lyse cyanobacterial cells, increasing extracellular cyanotoxin concentrations (AWWA and WRF, 2016). Methods to otherwise achieve compliance with the Disinfection Byproduct Rule (DBPR, 40 CFR Sections 141 and 142), such as increasing the pH (which decreases chlorine's rate of cyanotoxin oxidation) and using chloramines and chlorine dioxide (which do not degrade cyanotoxins well) may not be effective (AWWA and WRF, 2016). Therefore, chlorine disinfection must be optimized if intended to control cyanotoxins (Liu et al., 2020c).

5.3.2 Ultraviolet irradiation

UV irradiation has been demonstrated to decompose several cyanotoxins (Tsuji et al., 1995). However, the energies required to photolytically destroy these compounds are orders of magnitude greater than those needed for disinfection. Thus, UV treatment is not likely effective at inactivating cyanotoxins when the equipment is configured for conventional pathogen disinfection (AWWA and WRF, 2016). However, UV may be a viable option when used in conjunction with hydrogen peroxide to produce hydroxyl radicals for degrading MC, ATX-a, and CYN (Qiao et al., 2005; Song et al., 2009). Advanced oxidation using titanium dioxide in visible and UV light requires acidic conditions, making it a less feasible option at this time (Choi et al., 2007; Liu et al., 2003; Westrick et al., 2010).

5.3.3 Potassium permanganate

Each cyanotoxin reacts differently with potassium permanganate. MC-LR reacts moderately quickly and is not dependent on pH (Rodríguez et al., 2007a,b). Potassium permanganate reacts quickly with ATX-a, especially at pH 8–10 (Hall et al., 2000;

Rodriquez et al., 2007b). However, it does not effectively degrade CYN (Rodríguez et al., 2007b) or STX (Ho et al., 2009).

5.3.4 Ozone

Onstad et al., (2007) reported that ozonolysis effectively degrades MC independent of pH, and reactions with ATX-a depend on pH when pH values are 7–10, and reactions with CYN are pH dependent at levels of 4–10. However, STX is resistant to oxidation by ozone (Rositano et al., 2001).

5.4 Activated Carbon

There are two principal types of activated carbon used for removing organic compounds from drinking water. Powdered activated carbon (PAC) is temporarily added to water at the front of a treatment process to remove transient contaminants. Granular activated carbon (GAC) is used in fixed flow-through beds. The ability of PAC and GAC to remove extracellular cyanotoxins depends on the size of the cyanotoxin molecule, pH, and the presence of other organic matter that can compete with activated carbon adsorption sites (AWWA and WRF, 2016). Jar testing should be conducted when considering PAC treatment to optimize the dose. GAC can be used as both filter media and an adsorber, and rapid, small-scale column tests can be used to determine proper GAC column design. However, GAC removes cyanotoxins more effectively when used as an adsorber rather than a filter (Westrick et al., 2010). MC and CYN can be removed by activated carbon with high mesopore capacities, but with various degrees of efficiency (Ho et al., 2008; Newcombe, 2002). STX have a lower molecular mass and can be adsorbed by activated carbon with pores smaller than 1 nm (Ho et al., 2009). Vlad et al. (2019) suggested that GAC can be used to remove ATX-a, although additional study is required to understand its practical use in natural waters.

6 Texas Case Studies

There are few peer-reviewed studies specific to cyanobacterial blooms or cyanotoxins in Texas. Nevertheless, cyanobacteria occur widely throughout the state. For example, the US Geological Survey Texas Water Science Center (TXWSC) sampled 18 reservoirs, representing a variety of physicochemical conditions, and found cyanobacteria in all locations, as well as MCs in four reservoirs, CYN in two reservoirs, and STX in one reservoir (USGS, 2021a). During 2006 (USGS, 2008) and 2016-2019 (Trevino and Peterson, 2020), the US Geological Survey (USGS) and Texas Commission on Environmental Quality (TCEQ) used various quantitative and qualitative methods to survey up to 41 Texas waterbodies for cyanobacteria, cyanotoxins and taste-and-odor compounds. The TXWSC, the National Oceanic and Atmospheric Administration's (NOAA's) Harmful Algal BloomS Observing System (HABSOS, https://habsos.noaa.gov), the Lower Colorado River Authority (LCRA, https://www.lcra.org), and other municipal

authorities routinely monitor waterbodies for blooms in and around Texas and warn the public accordingly. Aside from public notifications on cyanotoxin blooms, many of the cyanotoxin reports in the popular press stem from the deaths of dogs and other animals. Animals can suffer neurological or gastrointestinal effects after consuming water contaminated with cyanotoxins or licking cyanobacteria from their fur. Since 1970, reports of animal deaths have increased markedly, including reports from Texas. However, it is unclear whether these case reports resulted from worsening water quality or increased public awareness. Most of these canine intoxication cases followed inhalation, ingestion, or dermal exposure to fresh water (Backer et al., 2013). Case studies of cyanobacterial blooms or cyanotoxin events in Texas are reported below.

6.1 Large Reservoir in West Texas, 2015–2017

Subbiah et al. (2019) studied a large reservoir in west Texas over the course of two years and found that the most commonly detected cyanotoxins were CYN and STX, while the least frequently detected were MCs. That study found that MC and ATX concentrations correlated directly with turbidity and total phosphorus, while CYN concentrations decreased with increased turbidity. Both CYN and STX were directly correlated with water temperature.

6.2 Buffalo Springs Lake and Lake Ransom Canyon, 2003–2004

Billam et al. (2006) studied MC-LR concentrations in Buffalo Springs Lake and Lake Ransom Canyon, in Lubbock, during 2003–2004. This study found the greatest MC-LR concentrations in both lakes during the spring. MC-LR concentrations correlated positively with dissolved oxygen and negatively with temperature and pH.

6.3 National Lakes Assessments, 2007, 2012, and 2017

The EPA National Lakes Assessments in 2007, 2012, and 2017 surveyed lakes across the country for cyanobacteria and cyanotoxins (USEPA, 2021). The 2007 study reported CYN, MC, and STX in various Texas lakes. One site contained NOD-R following greater-than-normal precipitation events and elevated salinity. This was an unexpected finding because NOD-R is normally associated with brackish waters, rather than inland fresh waters (Loftin et al., 2016). The 2012 study found a variety of cyanobacteria throughout Texas lakes. However, it reported cyanotoxins as MC equivalents using an Enzyme-Linked Immuno-Sorbent Assay (ELISA), and therefore did not list actual cyanotoxins (USEPA, 2012). The 2017 study similarly found various cyanobacteria throughout Texas but used ELISA tests for CYN and MC (USEPA, 2017).

6.4 Lady Bird Lake, 2019

Canine deaths were reported 30 minutes to 2 hours after swimming in Lady Bird Lake in Austin in August 2019. These deaths were attributed to ingesting dhATX, which was highly concentrated in metaphyton mats containing *Geitlerinema*, *Limnothrix*, *Pseudanabaena*, and *Phormidium*, as well as other bacteria, protozoa, and sediments. The floating mats appeared to have originated from benthic spires during eutrophic conditions and low hydrologic flows, and they remained until mid-November when cooler temperatures and increased water flows reduced the amount of cyanobacterial biomass (Manning et al., 2020). Lady Bird Lake, as well as Lake Austin, continue to be monitored for occurrences of cyanobacteria and cyanotoxins, and the city advises the public when to avoid allowing dogs to contact the water (www.austintexas.gov/page/algae).

6.5 Lake Travis, 2021

One dog died in January 2021, and four additional dogs became ill in late February 2021 after swimming in Lake Travis. There was also a winter storm with sub-freezing temperatures in mid-February. Water testing confirmed the presence of cyanobacteria and cyanotoxins, namely dhATX. This incident was unexpected, given the colder temperatures and relatively low turbidity (Dadamo, 2021). However, it is an example of the ability of cyanobacteria to produce cyanotoxins under a wide range of environmental conditions.

6.6 Lake Houston, 2006–2008

Beussink and Graham (2011) studied the relationships between hydrology and water quality, including cyanotoxins (MCs), in Lake Houston. In general, turbidity and nutrient concentrations were greatest during the cooler months (October to May) with higher water flows (residence times less than 100 days). Cyanobacteria were always present, although biovolume was greatest during the summer when temperatures were higher than 27°C and water residence times were longer than 400 days. These researchers explained that external nutrient loads to the reservoir were associated with suspended particles during inflow events, which increased turbidity and limited algal growth. Another factor that may have limited cyanobacterial bloom formation was the rapidly changing hydrology, which could stratify or destratify over several hours, thus preventing the extended periods of stable stratification often associated with cyanobacterial blooms.

6.7 Port Aransas Beach, 2007–2011

Yu (2011) studied cyanobacterial blooms from sandy intertidal beaches of the south Texas Gulf Coast near Port Aransas. *Hydrocoleum* and *Microcoleus* dominated the upper

layer of mature mats and exhibited substantial levels of nitrogenase activity. During dry seasons, subsurface cyanobacteria layers contained almost exclusively *Microcoleus*, but did not demonstrate nitrogenase activity. This study suggested that *Microcoleus* may serve as a foundation for intertidal sand mat formations, with *Hydrocoleum* providing structural integrity and nitrogen.

6.8 Lake Cliff Park and Other Dallas Waterbodies, 2017

The Dallas Park and Recreation office (2017) advised the public about a *Microcystis* bloom in Lake Cliff Park, as well as how to recognize and avoid it. This notice also advised the public on measures to help minimize blooms, such as removing dog waste, minimizing fertilizer use, using vegetation buffer strips for erosion, and notifying officials when they observe soil runoff.

6.9 Ingleside, 2016

In January 2016, Ingleside city officials advised approximately 200 residents to not drink and not boil water from their faucets after MC was detected in the drinking water system. Analyzing samples from throughout the affected distribution system revealed localized MC concentrations in the distribution system, but not the water provider's system. This finding, along with identification of unprotected cross-connections, indicated that the drinking water was contaminated by surface water during a cross-connection event. The City flushed the distribution system and installed new, reduced-pressure-zone backflow preventers, which reduced the localized MC concentrations but also spread the MC concentrations further throughout the distribution system. The City then responded by issuing a citywide "do not drink" order for children under the age of 6 and immunocompromised individuals. After three consecutive rounds of sampling with no detected MC, the City lifted the restrictions 13 days after the initial do not drink/do not boil order (AWWA, 2016; Hackleman, 2016; Sabawi, 2016).

6.10 Lake Texoma, 2011–2012

In 2011–2012, the Grayson County Health Department conducted 16-month epidemiological and water survey studies on cyanobacteria in Lake Texoma. Despite finding 75–125 cyanobacterial species in this waterbody, the only cyanotoxin found during this investigation was CYN, though not at concentrations of public health significance. The study also did not find reports of human or animal illness related to cyanotoxins in Lake Texoma (Teel et al., 2013).

6.11 Zebra Mussels

Given their propensity to cause environmental damage, including their ability to make conditions favorable for cyanobacteria, it is unlawful to possess or transport zebra

mussels, dead or alive, in Texas. Transporting this invasive species is punishable by a fine up to $500 per violation, with more serious punishments for repeat offenses. Zebra mussels were first sighted in Texas in 2009, at Lake Texoma (USGS, 2021b). Today, 24 lakes in Texas have been fully infested with zebra mussels, and they have been found in another nine lakes, as well as river reaches downstream of infested lakes. However, early and focused intervention at Lake Waco eradicated the zebra mussel, achieving a rare feat (Texas Parks and Wildlife Department, 2021).

7 Conclusions

Cyanobacteria and their toxins are gaining worldwide attention as they threaten the health and safety of animals and humans. Cyanobacteria have been found throughout water columns as planktonic species that can aggregate during blooms, as well as in the benthos as mats. Historically, they have been considered as algae, given their morphologies. However, morphology alone is insufficient to identify cyanobacteria, and phylogenetic molecular techniques have become the standard in identifying them. In fact, their taxonomy continues to evolve as understanding of their phylogeny improves.

Environmental factors, such as nutrient concentrations, light availability, temperature, waterbody hydrodynamics, pH, salinity, and other organisms, affect cyanobacteria growth. Although phosphorus is commonly regarded as rate limiting, nitrogen concentrations also can affect cyanobacteria success. Buoyancy, resistance to grazing, and overwintering strategies give cyanobacteria competitive advantages with regard to how they use resources such as nutrients and light, and these advantages frequently enable the cyanobacteria to survive conditions unfavorable to other microorganisms. However, cyanobacterial relationships with other organisms and environmental conditions are complex and waterbody specific.

Cyanobacteria strains that possess the appropriate genes produce cyanotoxins, and they can co-exist with nontoxic strains. The amount of cyanotoxin in a given clone can also vary, and seasonal patterns fluctuate among waterbodies. Environmental conditions are suspected of affecting whether these toxigenic genes are upregulated, but they are not yet well understood. Nevertheless, even if nontoxic clones dominate a given bloom, the toxic clones that are present may still produce toxins in excess of acceptable limits.

Cyanotoxins may present health concerns to humans when they are released from cyanobacterial cells, typically following cell lysis, and enter drinking water sources. Drinking water treatment efficacy varies among extracellular cyanotoxins, depending on their chemical and physical properties. Conventional drinking water treatment methods can remove intracellular (intact cyanobacteria) and extracellular cyanotoxins, although an integrated approach using multiple methods could be preferable.

Although there have been few peer-reviewed studies specific to cyanobacterial blooms or cyanotoxins in Texas, cyanobacteria have been observed widely throughout the state. Several regulatory authorities routinely monitor waterbodies for blooms in and around Texas and warn the public accordingly. Case studies in Texas largely involve animal deaths, when animals suffer neurological or gastrointestinal effects after consuming water contaminated with cyanotoxins or licking cyanobacteria from their fur. These incidents have raised concerns about cyanobacteria occurrences. Municipal authorities charged with controlling such occurrences must understand the features and patterns specific to each waterbody because these environmental factors are complex and dynamic.

8 References

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APPENDIX A

Short-Term Cyanotoxin Screening Levels

Appendix A: Short-Term Cyanotoxin Screening Levels

Under Task 1 of Work Order No.: 04 (PCR 23048: Comprehensive Literature Review of Cyanotoxin Toxicity and Health-Based Screening Level Derivation), ToxStrategies conducted a comprehensive literature review of toxicity factors for microcystins, cylindrospermopsin, and anatoxin-a (MCs, CYN, and ATX). Screening levels for drinking water are based on humans consuming drinking water (µg cyanotoxin per liter of water), and on animals consuming surface water (also µg cyanotoxin per liter of water). Animals can ingest intracellular cyanotoxins from cyanobacterial mats and crusts, and corresponding screening levels are also provided (mg cyanotoxin per kg of dry cyanobacteria). Existing regulatory, as well as candidate screening levels for MC-LR, CYN, and ATX for certain age groups, species, reference doses (RfD), exposure durations, and endpoints presented in the Task 1 report are given in Tables A1–A9. Refer to the Task 1 report for details on the derivation of these screening levels.

List of Tables

Table A1. Existing human health-based drinking water regulatory screening levels for cyanotoxins

Cyanotoxin*	Regulatory Agency	Type of Drinking Water Screening level	Description of Screening level	Drinking Water Screening level (µg/L)
Microcystins	USEPA (2015a)	10-Day Health Advisory	Children pre-school age and younger (under 6 years old); applied as total microcystins using microcystin-LR as a surrogate	0.3
Microcystins	USEPA (2015a)	10-Day Health Advisory	School-age children (6 years and older); applied as total microcystins using microcystin-LR as a surrogate	1.6
Microcystins	WHO (2020a)	Provisional Guideline Value; based on lifetime drinking water exposure	Microcystin-LR (free plus cell-bound microcystins) for adults lifetime exposure	1
Microcystins	WHO (2020a)	Provisional Guideline Value; based on short-term drinking water exposure (2 weeks)	Microcystin-LR (free plus cell-bound microcystins) for adults short-term exposure (up to 2 weeks)	12
Microcystins	Brazil, Uruguay, China, Czech Republic, Denmark, Germany, Italy, Japan, Korea, Netherlands, Norway, New Zealand, Poland, South Africa, Spain, France, Finland (WHO, 2017)	Drinking Water Guideline	Microcystin-LR (free plus cell-bound microcystins) for adults lifetime exposure	1
Microcystins	Australia, NHMRC, NRMMC (2011)	Drinking Water Guideline	Total microcystins expressed as microcystin-LR toxicity equivalents for lifetime exposure	1.3

Cyanotoxin*	Regulatory Agency	Type of Drinking Water Screening level	Description of Screening level	Drinking Water Screening level (µg/L)
Microcystins	Health Canada (2017)	Drinking Water Guideline	Total microcystins expressed as microcystin-LR toxicity equivalents; for seasonal exposure (<30 days)	1.5
Microcystins	Minnesota Department of Health (2015)	Guideline Value	Acute (1-day or less), Short-term (>1 day to 30 days), Subchronic (3- days to 10% of lifetime), and Chronic Non-Cancer Health Based Value	0.1
Microcystins	Oregon Health Authority (2019)	Drinking Water Guideline	Ages 5 years and younger; up to 10 days	0.3
Microcystins	Oregon Health Authority (2019)	Drinking Water Guideline	Adults; up to 10 days	1.6
Microcystins	Ohio EPA (2020)	Drinking Water Threshold	Do Not Drink – children under 6 and sensitive populations; up to 10 days	0.3
Microcystins	Ohio EPA (2020)	Drinking Water Threshold	Do Not Drink – children 6 and older and adults; up to 10 days	1.6
Cylindrospermopsin	USEPA (2015b)	10-Day Health Advisory	Children pre-school age and younger (under 6 years old)	0.7
Cylindrospermopsin	USEPA (2015b)	10-Day Health Advisory	School-age children (6 years and older)	3
Cylindrospermopsin	WHO (2020b)	Provisional lifetime drinking water health-based guidance value	Adult lifetime	0.7
Cylindrospermopsin	WHO (2020b)	Provisional Guideline Value; based on short-term exposure	Adult short-term (up to 2 weeks)	3.0

Cyanotoxin*	Regulatory Agency	Type of Drinking Water Screening level	Description of Screening level	Drinking Water Screening level (µg/L)
Cylindrospermopsin	Australia (2018)	Health Alert	Due to lack of adequate data, no guideline is set for cylindrospermopsin; however, an initial health alert is estimated	1
Cylindrospermopsin	New Zealand (2018)	Drinking Water Standard	Provisional maximum acceptable value; lifetime	1
Cylindrospermopsin	Brazil (2009)	Guideline for Drinking Water Quality (Recommended)	N/A	15
Cylindrospermopsin	Oregon Health Authority (2019)	Drinking Water Guideline	Ages 5 years and younger; up to 10 days	0.7
Cylindrospermopsin	Oregon Health Authority (2019)	Drinking Water Guideline	Adults; up to 10 days	3
Cylindrospermopsin	Ohio EPA (2020)	Drinking Water Threshold	Do Not Drink – children under 6 and sensitive populations; up to 10 days	0.7
Cylindrospermopsin	Ohio EPA (2020)	Drinking Water Threshold	Do Not Drink – children 6 and older and adults; up to 10 days	3.0
Anatoxin-a	USEPA (2015c)	No drinking water value	Unable to derive due to lack of data	N/A
Anatoxin-a	WHO (2020c)	Provisional short-term drinking water health-based reference value	Adults; up to 2 weeks	30
Anatoxin-a	New Zealand	Drinking Water Guideline	Provisional maximum acceptable value; lifetime	6
Anatoxin-a	Minnesota Department of Health (2016)	Risk Assessment Advice	Short-term Non-Cancer Risk Assessment Advice (>1 day to 30 days)	0.1

Cyanotoxin*	Regulatory Agency	Type of Drinking Water Screening level	Description of Screening level	Drinking Water Screening level (µg/L)
Anatoxin-a	Oregon Health Authority (2019)	Drinking Water Guideline	Ages 5 years and younger; up to 10 days	0.7
Anatoxin-a	Oregon Health Authority (2019)	Drinking Water Guideline	Adults; up to 10 days	3
Anatoxin-a	Ohio EPA (2020)	Drinking Water Threshold	Do Not Drink – children under 6 and sensitive populations; up to 10 days	0.3
Anatoxin-a	Ohio EPA (2020)	Drinking Water Threshold	Do Not Drink – children 6 and older and adults; up to 10 days	1.6

*Screening level applies to total cyanotoxins for the class (e.g., total microcystins), unless indicated differently in the Description of Screening level column.

Ohio EPA – Ohio Environmental Protection Agency, N/A – Not available, WHO – World Health Organization, USEPA – United States Environmental Protection Agency

Table A2. Existing dog and livestock regulatory screening levels for cyanotoxins

Cyanotoxin	Regulatory Agency	Description of Screening Level	Species*	Screening Level for Water Intake (µg/L)	Screening Level for Crust & Mat Consumption (mg/kg-dry weight)
Microcystins (Includes microcystins LA, LR, RR, and YR)	OEHHA (2012)	Acute (<24 hrs, exposure for a single day)	Dog	100	0.5
Microcystins (Includes microcystins LA, LR, RR, and YR)	OEHHA (2012)	Acute (<24 hrs, exposure for a single day)	Cattle	50	5
Microcystins (Includes microcystins LA, LR, RR, and YR)	OEHHA (2012)	Subchronic (up to 10% of lifetime)	Dog	2	0.01
Microcystins (Includes microcystins LA, LR, RR, and YR)	OEHHA (2012)	Subchronic (up to 10% of lifetime)	Cattle	0.9	1
Cylindrospermopsin	OEHHA (2012)	Acute (<24 hrs, exposure for a single day)	Dog	200	0.5
Cylindrospermopsin	OEHHA (2012)	Acute (<24 hrs, exposure for a single day)	Cattle	60	5
Cylindrospermopsin	OEHHA (2012)	Subchronic (up to 10% of lifetime)	Dog	10	0.04
Cylindrospermopsin	OEHHA (2012)	Subchronic (up to 10% of lifetime)	Cattle	5	0.4
Anatoxin-a	OEHHA (2012)	Acute (<24 hrs, exposure for a single day)	Dog	100	0.3
Anatoxin-a	OEHHA (2012)	Acute (<24 hrs, exposure for a single day)	Cattle	40	3
Anatoxin-a	OEHHA (2012)	Subchronic (up to 10% of lifetime)	Dog	100	0.3
Anatoxin-a	OEHHA (2012)	Subchronic (up to 10% of lifetime)	Cattle	40	3

* Cattle screening levels based on small breed dairy cow exposure scenario (average body weight of 454 kg). OEHHA - California EPA's Office of Environmental Health Hazard Assessment

Table A3. Candidate human health-based drinking water screening levels for microcystin-LR

Target Age Group	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Human Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Adult	2.6	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	1000 (UF_A = 10, UF_H = 10, UF_D = 10)	Relative liver weight	**83.2**	Chernoff et al. (2020)	Drinking water
Adult	0.43	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	43 (DAF = 7)	100 (UF_A = 1, UF_H = 10, UF_D = 10)	Liver histopathology (degeneration)	**13.8**	Mrdjen et al. (2018)	Drinking water
Adult	0.139	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	12.5 (DAF = 4)	90 (UF_A = 1, UF_H = 10, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**4.4**	Heinze (1999)	Drinking water
*USEPA HA adult**	*0.05*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*50 (LOAEL)*	*50 (no DAF)*	*1000 (UF_A = 10, UF_H = 10, UF_L = 3, UF_D = 3)*	*Liver toxicity*	***1.6***	*Heinze (1999)*	*Drinking water*
Child	2.6	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	1000 (UF_A = 10, UF_H = 10, UF_D = 10)	Relative liver weight	**17**	Chernoff et al. (2020)	Drinking water

Target Age Group	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Human Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Child	0.43	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	43 (DAF = 7)	100 (UF_A = 1, UF_H = 10, UF_D = 10)	Liver histopathology (degeneration)	**3**	Mrdjen et al. (2018)	Drinking water
Child	0.139	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	12.5 (DAF = 4)	90 (UF_A = 1, UF_H = 10, UF_L= 3, UF_D = 3)	Liver lesions (incl. degeneration)	**0.9**	Heinze (1999)	Drinking water
*USEPA HA child**	*0.05*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*50 (LOAEL)*	*50 (no DAF)*	*1000 (UF_A = 10, UF_H = 10, UF_L = 3, UF_D = 3)*	*Liver toxicity*	***0.3***	*Heinze (1999)*	*Drinking water*

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, incl. – including, LOAEL - lowest observed adverse effect level, mg/kg – milligram per kilogram, mg/kg-day – milligram per kilogram per day, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_H - intraspecies variability factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter, USEPA – US Environmental Protection Agency

Table A4. Candidate animal health-based drinking water screening levels for microcystin-LR

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Dogs	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1 UF_D = 10)	Mortality	**145**	Jackson et al. (1984)	
Dogs	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**102**	Chernoff et al. (2020)	
Dogs	5.7	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	57 (DAF = 5.3)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**22**	Mrdjen et al. (2018)	
Dogs	1.9	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	17 (DAF = 3)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**7.3**	Heinze (1999)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
*Dogs**	*37*	*1 day*	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1 UF_D = 10)*	*Mortality*	***100***	*Jackson et al. (1984)*	*OEHHA, based on acute RfD*
*Dogs**	*0.64*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*6.4 (BMDL)*	*6.4 (no DAF)*	*10 (UF_A = 3, UF_D = 3)*	*Liver toxicity*	***2***	*Heinze (1999)*	*OEHHA, based on subchronic RfD*
Dairy cattle	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1 UF_D = 10)	Mortality	**54**	Jackson et al. (1984)	
Dairy cattle	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**38**	Chernoff et al. (2020)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Dairy cattle	2.4	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	24 DAF = 12.4)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**3.5**	Mrdjen et al. (2018)	
Dairy cattle	0.79	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	7 (DAF = 7)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**1.2**	Heinze (1999)	
*Dairy cattle**	*37*	*1 day*	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1 UF_D = 10)*	*Mortality*	***50***	*Jackson et al. (1984)*	*OEHHA, based on acute RfD*
*Dairy cattle**	*0.64*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*6.4 (BMDL)*	*6.4 (no DAF)*	*10 (UF_A = 3, UF_D = 3)*	*Liver toxicity*	***0.9***	*Heinze (1999)*	*OEHHA, based on subchronic RfD*

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Beef cattle	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Mortality	**176**	Jackson et al. (1984)	
Beef cattle	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**124**	Chernoff et al. (2020)	
Beef cattle	2.4	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	24 DAF = 12.4)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**11.6**	Mrdjen et al. (2018)	
Beef cattle	0.79	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	7 (DAF = 7)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**3.8**	Heinze (1999)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
*Beef cattle**	*37*	*1 day*	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***200***	*Jackson et al. (1984)*	*OEHHA, based on acute RfD*
*Beef cattle**	*0.64*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*6.4 (BMDL)*	*6.4 (no DAF)*	*10 (UF_A = 3, U_{FD} = 3)*	*Liver toxicity*	***3***	*Heinze (1999)*	*OEHHA, based on subchronic RfD*
Horses	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Mortality	**206**	Jackson et al. (1984)	
Horses	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**144**	Chernoff et al. (2020)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Horses	2.6	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	26 (DAF = 11.8)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**14**	Mrdjen et al. (2018)	
Horses	0.84	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	7.6 (DAF = 6.6)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**5**	Heinze (1999)	

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, incl. – including, LOAEL - lowest observed adverse effect level, MC – microcystin, mg/kg – milligram per kilogram, mg/kg-day – milligram per kilogram per day, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

Table A5. Candidate animal mat/crust health-based screening levels for microcystin-LR

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Dogs	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Mortality	**0.5**	Jackson et al. (1984)	
Dogs	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**0.35**	Chernoff et al. (2020)	
Dogs	5.7	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	57 (DAF = 5.3)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**0.08**	Mrdjen et al. (2018)	
Dogs	1.9	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	17 (DAF = 3)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**0.025**	Heinze (1999)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
*Dogs**	*37*	*1 day*	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***0.5***	*Jackson et al. (1984)*	*OEHHA, based on acute RfD*
*Dogs**	*0.64*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*6.4 (BMDL)*	*6.4 (no DAF)*	*10 (UF_A = 3, UF_D = 3)*	*Liver toxicity*	***0.01***	*Heinze (1999)*	*OEHHA, based on subchronic RfD*
Dairy cattle	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Mortality	**5**	Jackson et al. (1984)	
Dairy cattle	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**3**	Chernoff et al. (2020)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Dairy cattle	2.4	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	24 DAF = 12.4)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**0.3**	Mrdjen et al. (2018)	
Dairy cattle	0.79	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	7 (DAF = 7)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver lesions (incl. degeneration)	**0.1**	Heinze (1999)	
Dairy cattle	*37*	*1 day*	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***5***	*Jackson et al. (1984)*	*OEHHA, based on acute RfD*
Dairy cattle	*0.64*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*6.4 (BMDL)*	*6.4 (no DAF)*	*10 (UF_A = 3, UF_D = 3)*	*Liver toxicity*	***0.1***	*Heinze (1999)*	*OEHHA, based on subchronic RfD*

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Beef cattle	37	1 day	Sheep	0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])	3700 (NOAEL)	3700 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Mortality	**6**	Jackson et al. (1984)	
Beef cattle	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10, UF_L = 1, UF_D = 10)	Relative liver weight	**5**	Chernoff et al. (2020)	
Beef cattle	2.4	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	24 DAF = 12.4)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**0.4**	Mrdjen et al. (2018)	
Beef cattle	0.79	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	7 (DAF = 7)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver toxicity Liver lesions (incl. degeneration)	**0.1**	Heinze (1999)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
*Beef cattle**	*37*	*1 day*	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***6***	*Jackson et al. (1984)*	*OEHHA, based on acute RfD*
*Beef cattle**	*0.64*	*28 days*	*Rat*	*0, 50, or 150 µg/kg-day*	*6.4 (BMDL)*	*6.4 (no DAF)*	*10 (UF_A = 3, UF_D = 3)*	*Liver toxicity*	***0.1***	*Heinze (1999)*	*OEHHA, based on subchronic RfD*
Horses	37	1 day	*Sheep*	*0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])*	*3700 (NOAEL)*	*3700 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	Mortality	**6.9**	Jackson et al. (1984)	
Horses	26	1 day	Mouse	0, 3, 5, 7 or 9 mg/kg	2600 (BMDL)	2600 (no DAF)	100 (UF_A = 10 UF_L = 1, UF_D = 10)	Relative liver weight	**4.8**	Chernoff et al. (2020)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Horses	2.6	7 days	Mouse	0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day	301 (BMDL)	26 (DAF = 11.8)	10 (UF_A = 1, UF_L = 1, UF_D = 10)	Liver histopathology (degeneration)	**0.5**	Mrdjen et al. (2018)	
Horses	0.84	28 days	Rat	0, 50, or 150 µg/kg-day	50 (LOAEL)	7.6 (DAF = 6.6)	9 (UF_A = 1, UF_L = 3, UF_D = 3)	Liver toxicity Liver lesions (incl. degeneration)	**0.16**	Heinze (1999)	

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, incl. – including, LOAEL - lowest observed adverse effect level, MC – microcystin, mg/kg – milligram per kilogram, mg/kg-day – milligram per kilogram per day, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

Table A6. Candidate human health-based drinking water screening levels for cylindrospermopsin

Target Age Group	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Human Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Adult	0.085	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	2.6 (DAF = 7)	30 (UF_A = 1, UF_H = 10, UF_L = 1 UF_D = 3)	Relative Kidney weight	**2.7**	Humpage & Falconer (2003)	Drinking water
*USEPA HA adult**	*0.10*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*30 (NOAEL)*	*30 (no DAF)*	300 (UF_A = 10, UF_H = 10, UF_L = 1 UF_D = 3)	*Kidney toxicity*	***3.2***	*Humpage & Falconer (2003)*	*Drinking water*
Child	0.085	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	2.6 (DAF = 7)	30 (UF_A = 1, UF_H = 10, UF_L = 1 UF_D = 3)	Relative Kidney weight	**0.6**	Humpage & Falconer (2003)	Drinking water
*USEPA HA child**	*0.10*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*30 (NOAEL)*	*30 (no DAF)*	300 (UF_A = 10, UF_H = 10, UF_L = 1 UF_D = 3)	*Kidney toxicity*	***0.7***	*Humpage & Falconer (2003)*	*Drinking water*

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, NOAEL - no observed adverse effect level, RfD – reference dose, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_H - intraspecies variability factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter, USEPA – US Environmental Protection Agency

Table A7. Candidate animal health-based drinking water screening levels for cylindrospermopsin

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Dogs	40	1 day	Mouse	4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)	4000 (NOAEL)	4000 (no DAF)	100 (UF_A = 10, UF_L = 1 UF_D = 10)	Mortality	**157**	Shaw et al. (2000) & Seawright et al. (1999)	
Dogs	3.4	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	3.4 (DAF = 5.3)	1 (UF_A = 1, UF_L = 1 UF_D = 1)	Relative Kidney weight	**13**	Humpage & Falconer (2003)	
*Dogs**	*40*	*1 day*	*Mouse*	*4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)*	*4000 (NOAEL)*	*4000 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D and severity of endpoint = 10)*	*Mortality*	***200***	*Shaw et al. (2000) & Seawright et al. (1999)*	*OEHHA, based on acute RfD*
*Dogs**	*3.3*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*33 (BMDL)*	*33 (no DAF)*	*10 (UF_A = 3, UF_L = 1, UF_D = 3)*	*Kidney toxicity*	***10***	*Humpage & Falconer (2003)*	*OEHHA, based on subchronic RfD*
Dairy cattle	24	13 weeks	Mouse	0, 75, 150, or 300 µg/kg-day	300 (NOAEL)	24 (DAF = 12.4)	*1 (UF_A = 1, UF_L = 1, UF_D = 1)*	Mortality	**35**	Chernoff et al. (2018)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Dairy cattle	1.4	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	1.4 (DAF = 12.4)	*1 (UF_A = 1, UF_L = 1, UF_D = 1)*	Relative Kidney weight	**2.1**	Humpage & Falconer (2003)	
*Dairy cattle**	*40*	*1 day*	*Mouse*	*4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)*	*4000 (NOAEL)*	*4000 (no DAF)*	*100 (UF_A = 10, UF_L = 1 UF_D = 10)*	*Mortality*	***60***	*Shaw et al. (2000) & Seawright et al. (1999)*	*OEHHA, based on acute RfD*
*Dairy cattle**	*3.3*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*33 (BMDL)*	*33 (no DAF)*	*10 (UF_A = 3, UF_L = 1, UF_D = 3)*	*Kidney toxicity*	***5***	*Humpage & Falconer (2003)*	*OEHHA, based on subchronic RfD*
Beef cattle	24	13 weeks	Mouse	0, 75, 150, or 300 µg/kg-day	300 (NOAEL)	24 (DAF = 12.4)	*1 (UF_A = 1, UF_L = 1, UF_D = 1)*	Mortality	**115**	Chernoff et al. (2018)	
Beef cattle	1.4	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	1.4 (DAF = 12.4)	*1 (UF_A = 1, UF_L = 1, UF_D = 1)*	Relative Kidney weight	**6.9**	Humpage & Falconer (2003)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
*Beef cattle**	*40*	*1 day*	*Mouse*	*4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)*	*4000 (NOAEL)*	*4000 (no DAF)*	*100 (UF_A = 10, UF_L = 1 UF_D = 10)*	*Mortality*	***200***	*Shaw et al. (2000) & Seawright et al. (1999)*	*OEHHA, based on acute RfD*
*Beef cattle**	*3.3*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*33 (BMDL)*	*33 (no DAF)*	*10 (UF_A = 3, UF_L = 1, UF_D = 3)*	*Kidney toxicity*	***20***	*Humpage & Falconer (2003)*	*OEHHA, based on subchronic RfD*
Horses	25	13 weeks	Mouse	0, 75, 150, or 300 µg/kg-day	300 (NOAEL)	25 (DAF = 11.8)	*1 (UF_A = 1, UF_L = 1, UF_D = 1)*	Mortality	**141**	Chernoff et al. (2018)	
Horses	1.5	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	1.5 (DAF = 11.8)	*1 (UF_A = 1, UF_L = 1, UF_D = 1)*	Relative Kidney weight	**8.4**	Humpage & Falconer (2003)	

* Regulatory screening levels are indicated with an asterisk and blue, italicized text .

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, mg/kg – milligram per kilogram, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

Table A8. Candidate animal mat/crust health-based screening levels for cylindrospermopsin

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Dogs	40	1 day	Mouse	4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)	4000 (NOAEL)	4000 (no DAF)	100 (UF_A = 10, UF_L = 1 UF_D = 10)	Mortality	**0.53**	Shaw et al. (2000) & Seawright et al. (1999)	
Dogs	3.4	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	3.4 (DAF = 5.3)	1 (UF_A = 1, UF_L = 1 UF_D = 1)	Relative Kidney weight	**0.045**	Humpage & Falconer (2003)	
*Dogs**	*40*	*1 day*	*Mouse*	*4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)*	*4000 (NOAEL)*	*4000 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D and severity of endpoint = 10)*	*Mortality*	***0.53***	*Shaw et al. (2000) & Seawright et al. (1999)*	*OEHHA, based on acute RfD*
*Dogs**	*3.3*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*33 (BMDL)*	*33 (no DAF)*	*10 (UF_A = 3, UF_L = 1, UF_D = 3)*	*Kidney toxicity*	***0.044***	*Humpage & Falconer (2003)*	*OEHHA, based on subchronic RfD*

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Dairy cattle	24	13 weeks	Mouse	0, 75, 150, or 300 µg/kg-day	300 (NOAEL)	24 (DAF = 12.4)	1 (UF_A = 1, UF_L = 1, UF_D = 1)	Mortality	**3.1**	Chernoff et al. (2018)	
Dairy cattle	1.4	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	1.4 (DAF = 12.4)	1 (UF_A = 1, UF_L = 1, UF_D = 1)	Relative Kidney weight	**0.19**	Humpage & Falconer (2003)	
*Dairy cattle**	*40*	*1 day*	*Mouse*	*4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)*	*4000 (NOAEL)*	*4000 (no DAF)*	*100 (UF_A = 10, UF_L = 1 UF_D = 10)*	*Mortality*	***5.1***	*Shaw et al. (2000) & Seawright et al. (1999)*	*OEHHA, based on acute RfD*
*Dairy cattle**	*3.3*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*33 (BMDL)*	*33 (no DAF)*	*10 (UF_A = 3, UF_L = 1, UF_D = 3)*	*Kidney toxicity*	***0.42***	*Humpage & Falconer (2003)*	*OEHHA, based on subchronic RfD*
Beef cattle	24	13 weeks	Mouse	0, 75, 150, or 300 µg/kg-day	300 (NOAEL)	24 (DAF = 12.4)	1 (UF_A = 1, UF_L = 1, UF_D = 1)	Mortality	**4.2**	Chernoff et al. (2018)	
Beef cattle	1.4	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	1.4 (DAF = 12.4)	1 (UF_A = 1, UF_L = 1, UF_D = 1)	Relative Kidney weight	**0.25**	Humpage & Falconer (2003)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
*Beef cattle**	*40*	*1 day*	*Mouse*	*4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)*	*4000 (NOAEL)*	*4000 (no DAF)*	*100 (UF_A = 10, UF_L = 1 UF_D = 10)*	*Mortality*	***7.0***	*Shaw et al. (2000) & Seawright et al. (1999)*	*OEHHA, based on acute RfD*
*Beef cattle**	*3.3*	*11 weeks*	*Mouse*	*0, 30, 60, 120, or 240 µg/kg-day*	*33 (BMDL)*	*33 (no DAF)*	*10 (UF_A = 3, UF_L = 1, UF_D = 3)*	*Kidney toxicity*	***0.58***	*Humpage & Falconer (2003)*	*OEHHA, based on subchronic RfD*
Horses	25	13 weeks	Mouse	0, 75, 150, or 300 µg/kg-day	300 (NOAEL)	25 (DAF = 11.8)	1 (UF_A = 1, UF_L = 1, UF_D = 1)	Mortality	**4.7**	Chernoff et al. (2018)	
Horses	1.5	11 weeks	Mouse	0, 30, 60, 120, or 240 µg/kg-day	17.9 (BMDL)	1.5 (DAF = 11.8)	1 (UF_A = 1, UF_L = 1, UF_D = 1)	Relative Kidney weight	**0.28**	Humpage & Falconer (2003)	

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, mg/kg – milligram per kilogram, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

Table A9. Candidate human health-based drinking water screening levels for anatoxin-a and dihydroanatoxin-a

Target Age Group	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Human Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Adult	0.25	1 day (dhATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	10,000 (UF_A = 10, UF_H = 10, UF_L = 10, UF_D = 10)	Mortality	**8**	Puddick et al. (2021)	Drinking water
Adult	1.1	1 day (ATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	10,000 (UF_A = 10, UF_H = 10, UF_L = 10, UF_D = 10)	Mortality	**34**	Puddick et al. (2021)	Drinking water
*USEPA HA adult**	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	***No Value***	*NA*	*Drinking water*
Child	0.25	1 day (dhATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	10,000 (UF_A = 10, UF_H = 10, UF_L = 10, UF_D = 10)	Mortality	**1.7**	Puddick et al. (2021)	Drinking water
Child	1.1	1 day (ATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	10,000 (UF_A = 10, UF_H = 10, UF_L = 10, UF_D = 10)	Mortality	**7**	Puddick et al. (2021)	Drinking water
*USEPA HA child**	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	***No Value***	*NA*	*Drinking water*

* Regulatory screening levels are indicated with an asterisk and blue, italicized text .

ATX-a anatoxin-a, BMDL – benchmark dose level, DAF – dosimetric adjustment factor, dhATX-a – dihydroanatoxin-a , HA – health advisory, LD_{50} – lethal dose for 50 percent of the population, NA – not applicable, OECD – Organisation for Economic Co-operation and Development, RfD – reference dose, UF_A – interspecies uncertainty factor from animal to human, UF_D – database uncertainty factor, UF_H – intraspecies variability factor, UF_L – LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter, USEPA – US Environmental Protection Age

Table A10. Candidate animal health-based drinking water screening levels for anatoxin-a and dihydroanatoxin-a

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Dogs	2.5	1 day (dhATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**10**	Puddick et al. (2021)	
Dogs	11	1 day (ATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**42**	Puddick et al. (2021)	
*Dogs**	*25*	*5 day (ATX-a)*	*Mouse*	*1.2 – 12.3^ mg/kg-day*	*2500^ (NOAEL)*	*2500 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***98***	*Fawell (1999b)*	*OEHHA, acute and subchronic*
Dairy cattle	2.5	1 day (dhATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**3.6**	Puddick et al. (2021)	
Dairy cattle	11	1 day (ATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**15**	Puddick et al. (2021)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
*Dairy cattle**	*25*	*5 day (ATX-a)*	*Mouse*	*1.2 – 12.3^ mg/kg-day*	*2500^ (NOAEL)*	*2500 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***36***	*Fawell (1999b)*	*OEHHA, acute and subchronic*
Beef cattle	2.5	1 day (dhATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**12**	Puddick et al. (2021)	
Beef cattle	11	1 day (ATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**50**	Puddick et al. (2021)	
*Beef cattle**	*25*	*5 day (ATX-a)*	*Mouse*	*1.2 - 12.3^ mg/kg-day*	*2500^ (NOAEL)*	*2500 (no DAF)*	*100 (UF_A = 10, UF_L = 1, UF_D = 10)*	*Mortality*	***119***	*Fawell (1999b)*	*OEHHA, acute and subchronic*
Horses	2.5	1 day (dhATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**14**	Puddick et al. (2021)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (µg/L)	Study	Notes
Horses	11	1 day (ATX-a)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**59**	Puddick et al. (2021)	

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

^Doses are listed as reported by OEHHA (2012). However, doses in the reference study (Fawell 199b) are indicated as 1.5, 3, 7.5 or 15 mg/kg-day, with 3 mg/kg-day as the maximum tolerated dose.

ATX-a anatoxin-a, DAF – dosimetric adjustment factor, dhATX-a - dihydroanatoxin-a , LD_{50} - lethal dose for 50 percent of the population, mg/kg-day – milligram per kilogram per day, NOAEL – no observed adverse effect level, RfD – reference dose, OECD - Organisation for Economic Co-operation and Development, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

Table A11. Candidate animal mat/crust health-based screening levels for anatoxin-a and dihydroanatoxin-a

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
Dogs	2.5	1 day (dhATX)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**0.033**	Puddick et al. (2021)	
Dogs	11	1 day (ATX)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**0.14**	Puddick et al. (2021)	
*Dogs**	*25*	*5 day (ATX)*	*Mouse*	*1.2 - 12.3^ mg/kg-day*	*2500^ (NOAEL)*	*2500 (no DAF)*	100 (UF_A = 10, UF_L = 1, UF_D = 10)	*Mortality*	***0.33***	*Fawell (1999b)*	*OEHHA, acute and subchronic*
Dairy cattle	2.5	1 day (dhATX)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**0.32**	Puddick et al. (2021)	
Dairy cattle	11	1 day (ATX)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**1.4**	Puddick et al. (2021)	

Target Species	Candidate RfD (µg/kg-day)	Study Duration	Study Species	Administered Doses	Point of Departure (µg/kg-day)	Species Equivalent Dose (µg/kg-day)	Uncertainty Factors	Endpoint	Candidate Screening Level (mg/kg, dry weight)	Study	Notes
*Dairy cattle**	*25*	*5 day (ATX)*	*Mouse*	*1.2 - 12.3^ mg/kg-day*	*250^0 (NOAEL)*	*2500 (no DAF)*	100 (UF_A = 10, UF_L = 1, UF_D = 10)	*Mortality*	***3.2***	*Fawell (1999b)*	*OEHHA, acute and subchronic*
Beef cattle	2.5	1 day (dhATX)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**0.44**	Puddick et al. (2021)	
Beef cattle	11	1 day (ATX)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**1.9**	Puddick et al. (2021)	
*Beef cattle**	*25*	*5 day (ATX)*	*Mouse*	*1.2 - 12.3^ mg/kg-day*	*2500^ (NOAEL)*	*2500 (no DAF)*	100 (UF_A = 10, UF_L = 1, UF_D = 10)	*Mortality*	***4.4***	*Fawell (1999b)*	*OEHHA, acute and subchronic*
Horses	2.5	1 day (dhATX)	Mouse	Range; using OECD (2008) "up and down" procedure	2500 (LD_{50})	2500 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**0.5**	Puddick et al. (2021)	
Horses	11	1 day (ATX)	Mouse	Range; using OECD (2008) "up and down" procedure	10,600 (LD_{50})	10,600 (no DAF)	1000 (UF_A = 10, UF_L = 10, UF_D = 10)	Mortality	**2.0**	Puddick et al. (2021)	

* Regulatory screening levels are indicated with an asterisk and blue, italicized text .

^Doses are listed as reported by OEHHA (2012). However, doses in the reference study (Fawell 199b) are indicated as 1.5, 3, 7.5 or 15 mg/kg-day, with 3 mg/kg-day as the maximum tolerated dose.

ATX-a anatoxin-a, DAF – dosimetric adjustment factor, dhATX-a - dihydroanatoxin-a , LD_{50} - lethal dose for 50 percent of the population, mg/kg-day – milligram per kilogram per day, NOAEL – no observed adverse effect level, RfD – reference dose, OECD - Organisation for Economic Co-operation and Development, UF_A - interspecies uncertainty factor from animal to human, UF_D - database uncertainty factor, UF_L - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter