Intervention for microcystin-producing cyanobacteria and microcystins in freshwater resources: Development of a decision support document for risk management

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Executive summary

Microcystin (MC) production by cyanobacteria (i.e., blue-green algae) in freshwater resources has resulted in significant financial losses and adverse effects on the health of humans, pets, fish, wildlife, livestock, and plants. As harmful blooms of cyanobacteria (often referred to as harmful algal blooms, or "HABs") increase in frequency, intensity, and severity in freshwater systems throughout the United States and globally, the management decision of "no action" (or a decision not to intervene) results in loss of the beneficial services provided directly and indirectly by the water resource, and increases the likelihood that people and other organisms will be exposed to MCs. Peer-reviewed data and other useful information are readily available regarding characteristics of microcystins, effects on human health, ecological risks, and management approaches for MC-producing cyanobacteria and MCs. However, these data are rarely in a form that can be readily used to make scientifically defensible decisions. The goal of this paper was to provide a review of the literature related to risk management of MCs and to organize this information in a logical manner to provide a decision support document for water resource managers, regulators, and stakeholders.

Exposure influences risk, so properties of MC exposures are characterized in terms of source, chemical structures, environmental and toxicological properties, spatial and temporal distribution, and forms (cellular and aqueous). Potential human exposure routes are then described and ranked in terms of their importance (i.e., routes more likely to result in significant exposures). We also present a species sensitivity distribution (SSD) for mammals, birds, fish, aquatic invertebrates, and plants, which was developed by reviewing ecological toxicity data. We use these exposure and response data to compare potential outcomes resulting from no-action, exposure avoidance, and control from a high level. Following these comparisons, we describe the relative effectiveness, availability, durability, and scalability of long-term and short-term risk management approaches for MC-producing cyanobacteria and MCs based on peer-reviewed data, and lastly define and describe adaptive water resource management in this context.

The goal of this decision support document was to provide vetted and assembled information to aid in site-specific decision making and development of adaptive water resource management plans. There are still data gaps (particularly regarding human health), but our review reveals that sufficient information is available to effectively and efficiently manage risks associated with MC-producing cyanobacteria and MCs. With public awareness, stakeholder support, and persistent efforts, unnecessary exposures to MCs can be minimized or avoided, critical uses of freshwater resources can be maintained, and significant financial losses can be prevented.

Foreword

This document presents information gained from a thorough and strategic literature review and assembly of defensible data and information necessary to inform risk management decisions for microcystins and microcystin-producing cyanobacteria. Each objective of the study is separated into sections, and each section is preceded with a summary of the take-home messages from that section. The material discussed within each section is technical in nature to provide the scientific "back-bone" for those interested in the original data and studies used to assemble this document. The appendix at the end of the document includes tables with supplemental data referenced throughout the text.

Glossary of terms and abbreviations

MC: Abbreviation used for microcystin throughout the text

TDI: Tolerable daily intake; the dose of a substance taken in per day that is considered safe (i.e., not a health risk) to the specified receptor

NOEC: No observable effects concentration; the highest concentration of an exposure that resulted in no measurable adverse effects (i.e., no statistical difference from untreated control) to the receptor in a laboratory toxicity experiment (calculated based on hypothesis testing)

LOEC: Lowest observable effects concentration; the lowest exposed concentration of a substance that resulted in measurable adverse effects in a laboratory toxicity experiment (calculated based on hypothesis testing)

LC25 (LC = lethal concentration): The concentration of an exposure resulting in 25% mortality of the exposed population in a laboratory toxicity experiment

LC50 (LC = lethal concentration): The concentration of an exposure resulting in 50% mortality of the exposed population in a laboratory toxicity experiment

EC50 (EC = effects concentration): The concentration of an exposure that induces a response that is approximately 50%, or half, of the maximum measured response in a laboratory toxicity experiment

WHO: World Health Organization

USEPA: United States Environmental Protection Agency

SSD: Species Sensitivity Distribution

Table of contents

1 Introduction

Toxin production by cyanobacteria in freshwater resources has resulted in significant financial losses and adverse effects on the health of humans, pets, fish, wildlife, and plants. Common examples include the 2014 public water supply shutdown in Toledo, OH (Steffen et al., 2017) and beach closures in several Florida counties following transport of toxin-producing cyanobacteria from Lake Okeechobee downstream where they subsequently grew (Rosen et al., 2017). There have also been numerous reports of livestock (Galey et al., 1987; Puschner et al., 1998; Haynie et al., 2013) and domestic pet (Wood et al., 2010a; Lurling and Faassen, 2013) illnesses and mortalities following ingestion of cyanobacteria scums or infested water. Microcystins (MCs), a prevalent and potent group of toxins produced by cyanobacteria in freshwaters (Funari and Testai, 2008; Cheung et al., 2013; Wood, 2016), are frequently responsible for rendering drinking and recreational waters unusable, and for poisonings of humans, domestic animals, and wildlife. When a cyanobacterial bloom is noticed and public alarm is raised, water resource managers often prevent public use of the affected water resource due to lack of scientific information available to them, inexperience with MC-producing cyanobacteria, or failure to adequately consider the consequences of limiting public access.

As exposures to MCs in freshwater resources become more frequent, no decision (or a decision not to intervene) results in loss of services provided by the water resource, and increased exposures of people and other organisms to MCs that cannot or do not avoid exposures (e.g. Toledo's public water supply shutdown). The peer-reviewed literature includes information regarding exposures of MCs (e.g., source, forms, spatial and temporal distribution) and effects (human health and ecological risks), as well as management methods for MC-producing cyanobacteria, MCs, or both. However, these data are rarely vetted for quality assurance and quality control and are not always assembled in a way that is useful for making a scientifically defensible decision. The goal of this paper is to assemble and organize the information necessary for risk management of MCs and thereby provide a decision support document for water resource managers, regulators, and stakeholders.

The approach for assembling this information was based on the fundamental toxicological principle that exposures drive risks; thus, a first and foremost need is a thorough and accurate characterization of exposures (Figure 1). Exposures of MCs are influenced by their source (i.e., species of cyanobacteria), their intrinsic chemical structures and properties, spatial distribution, temporal distribution (i.e., duration and frequency), and form (cellular or aqueous). Another crucial factor is route of exposure; exposures drive responses, so specific exposure routes and effects can be parsed among humans and other organisms (mammals, fish, aquatic invertebrates, birds, and plants) (Figure 1). For human health, the relative importance of exposure routes in terms of potential for exposures can be ranked based on possible daily intake of MCs via different routes. An additional consideration is the potential for a complete exposure pathway, here defined as a measured exposure, resulting in a dose and a measured response, producing measurable adverse effects. If data are unavailable to confirm exposure pathways, potential for risks can be evaluated based on available evidence including exposure data and reported symptoms in humans following suspected exposures via different routes.

Regarding ecological risks of MCs, exposure-response relationships for individual species of animals and plants can be evaluated for effects thresholds and potencies, which would be especially important for keystone or endangered species. Overall, a species sensitivity distribution (SSD) can be assembled for MCs based on toxicological data for animals and plants. SSDs provide information regarding the relative sensitivities among species, the lower threshold for effects (i.e., at what concentration of MCs are effects observed for the most sensitive organisms that have been evaluated), the relative potency among species (i.e., proportion of total species affected with incremental increases in exposure concentration of MCs), and the distance between the lower and upper thresholds (i.e., the range in exposure concentrations of MCs from protective to catastrophic).

Additional information needed to trigger a management decision includes comparisons of the relative risks of no action and action (Figure 1). In this context, "no action" is operationally defined as unabated growth of cyanobacteria and production of MCs in a water resource, whereas action can include exposure avoidance or control (i.e., management). Both types of action can be compared to "no action" in terms of relative consequences, including effects and costs. Following these comparisons, we review and compare specific approaches for risk management based on management target or goals (i.e., MC-producing cyanobacteria or MCs) to provide information on relative effectiveness for different scenarios. Finally, the process of adaptive water resource management is defined for this context, and examples are provided. Ultimately, the purpose of this decision support system is to provide the information necessary to appropriately

intervene in MC exposures, to be adaptable as more data become available, and to provide site specificity for a range of situations that are frequently encountered.

The overall objective of this study was to assemble and organize information necessary to drive risk management for MCs as a decision support document. Specific objectives were to: 1) characterize exposures of MCs (in terms of source, chemical structures, environmental fate and toxicological properties, spatial and temporal distribution, and cellular and aqueous forms); 2) characterize potential human and ecological exposure routes and effects; 3) compare the relative risks of no action vs. action in terms of potential outcomes; 4) review currently available risk management approaches for MC-producing cyanobacteria and MCs; and 5) define and describe adaptive water resource management in the context of risk intervention for MC-producing cyanobacteria and MCs.

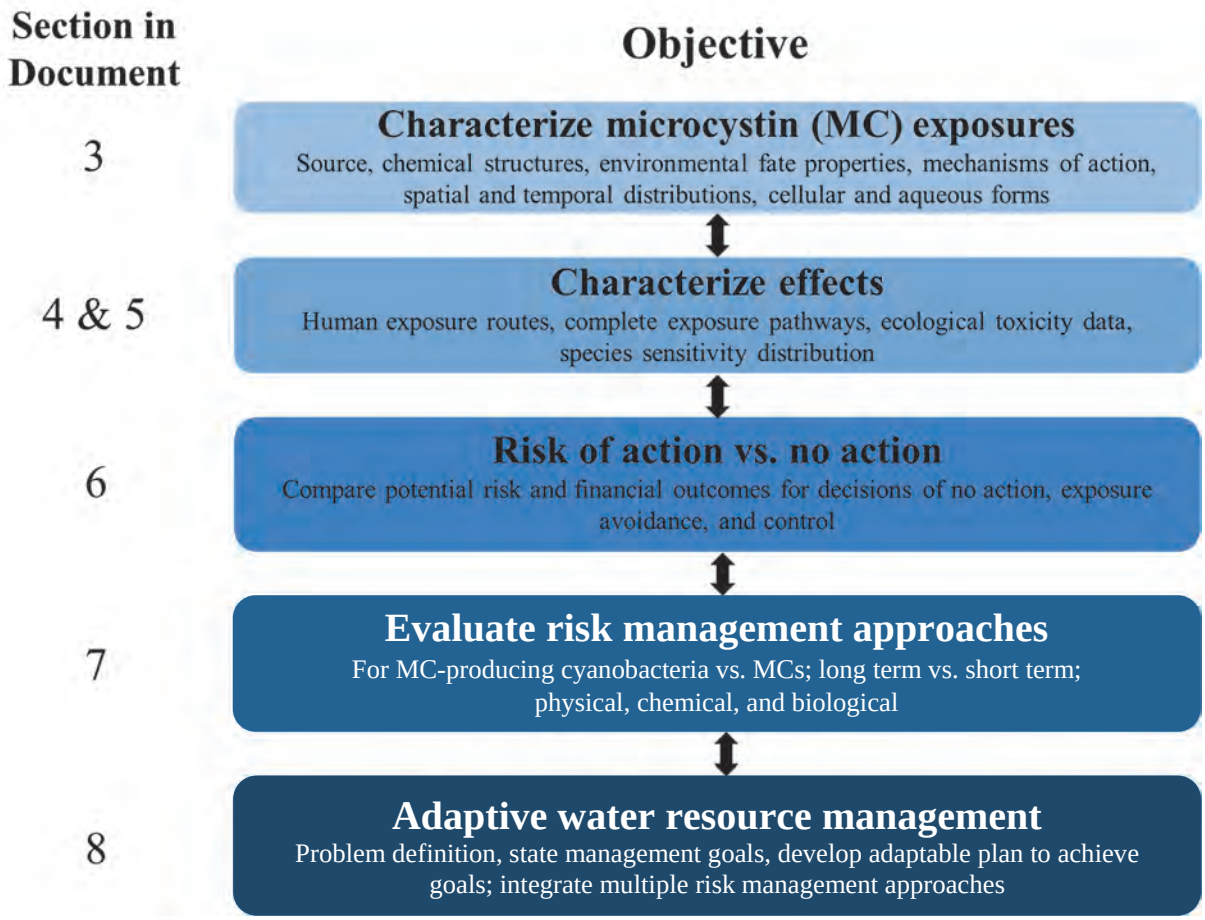

Figure 1. Conceptual model for objectives in this paper

2 Approach for development of document

2.1 Exposures of microcystins

Peer-reviewed articles in scientific journals and books, as well as "gray literature" (e.g., technical reports, government documents, and white papers), were sourced for all information in this paper. Exposures of MCs were characterized in terms of source, chemical structures, fate properties, mechanism of action, spatial distribution, temporal distribution, and forms (cellular and aqueous). Following this information, current regulations regarding exposures of MCs in drinking and recreational waters are discussed.

2.2 Potential human exposure routes for microcystins

Human exposure routes are parsed as direct and indirect exposures in this paper. Direct exposures include ingestion of drinking water, incidental ingestion of water during swimming or recreation, dermal contact during bathing or swimming, inhalation of aerosolized particles during bathing or swimming, and intravenous exposures during medical treatments. Indirect exposures are defined as ingestion of MCs that have bioconcentrated or bioaccumulated in food sources, including vegetables, fruits, grains, fish, and shellfish, and blue-green algal supplements (BGAS). For each exposure route, studies that reported both extraction and analytical techniques for MC measurements were included. All studies reviewed for this paper were identified using strategic literature reviews based on criteria for primary or secondary evidence as described below. For plant-based food exposures (e.g. vegetables, fruit, grains), studies that reported MC concentrations in edible portions of plants following known exposures to MCs (via irrigation) constituted primary evidence. Studies in which MC concentrations were measurable in plant-based food items, but exposure concentrations from irrigation waters were unknown, or studies that measured MC concentrations in portions of plants not used for human consumption (i.e., seedlings) constituted secondary evidence. For fish and shellfish food exposures, studies that reported measured MC concentrations in muscle or whole bodies of organisms collected directly from water resources were considered primary evidence, since these are realistic exposures for humans. In contrast, aquatic organisms in laboratory studies are frequently exposed to unrealistically high concentrations to elicit adverse responses, or unrealistic exposure routes have been used (i.e., intraperitoneal injections), thus bioconcentration or bioaccumulation of MCs in these lab-tested organisms may not be realistic.

Potential daily human exposures to MCs from ingestion (via drinking water, swimming water, and foods) and inhalation were estimated and contrasted with the tolerable daily intake (TDI) proposed by the World Health Organization (WHO) of 0.04 µg MC per kg of body weight (WHO, 2003). TDIs were calculated for two age groups (birth to less than 6 years of age; 6 years of age and greater including adult) because the greatest difference in average ingestion of water, fruits, vegetables, fish, and shellfish occurs between these groups (USEPA, 2011; USEPA, 2015). Body weights selected for TDI calculations were the highest of the average weights among each age group provided by the USEPA (2011). It should be noted that the WHO's TDI was calculated based on a conservative toxicity threshold from a laboratory toxicity study using mice, which was then lowered by a factor of 1000 due to several uncertainties in the data base (referred to as uncertainty factors). Therefore, the guidelines currently used for human health are extremely conservative and thus, the goal of providing estimates of possible exposures and exceedance of TDIs was not to support the conclusion that there is potential for risk based on these calculations. Rather, the goal was to parse which exposure routes are more likely to result in significant concentrations of MCs taken in by humans, to provide context for where efforts should be focused for risk management, and for future contribution of data for risk assessments.

2.3 Ecological toxicity

For ecological toxicity, studies were also designated as primary or secondary evidence. Criteria for selection of primary peer-reviewed studies for ecological toxicity data were: 1) exposures were measured (and analytical methods were specified and appropriate); 2) age or life stage of organisms was reported; 3) ecologically relevant response endpoints were measured (i.e., growth, survival, and/or reproduction); and 4) some toxicological value was (or could be) calculated based on measurements (e.g., NOEC, LOEC, LC50, EC50). If one or more criteria were not met, studies were not included in the species sensitivity distribution (SSD), which was assembled using the United States Environmental Protection Agency (USEPA) SSD generator (CADDIS v.4). The purpose of the SSD generated for this study was to provide a relative ranking of toxicity thresholds for plants and animals based on the strategic literature review criteria

(described above). Studies that reported adverse effects in organisms following a suspected exposure (e.g., post-hoc studies) were considered secondary evidence, but were not included in the SSD.

2.4 Risk of action vs. risk of no action

No action and action decisions were compared at a high level in terms of their outcomes, which included effects (summarized from preceding effects sections) and costs. No action was operationally defined in this study as unabated growth of cyanobacteria and production of MCs. Action decisions include exposure avoidance and control. Exposure avoidance was operationally defined as closure of water resources for drinking water or recreation in an effort to prevent human exposures. Control was defined as techniques used in isolation or combination that result in timely and substantial decreases in MC-producing cyanobacteria and/or MCs to levels that alleviate an existing or potential impairment to the uses or functions of the water resource (adapted from Netherland and Schardt, 2012). Outcomes contrasted among no action, exposure avoidance, and control included potential exposures and effects based on available evidence, and types of financial impacts.

2.5 Available approaches for risk management

Approaches for risk management were parsed as long-term and short-term options. In this context, long-term approaches are those applied to large scales (e.g., watershed) over the course of decades or centuries. Short-term approaches are applied locally (e.g., lake, reservoir, portion of lake or reservoir, or drinking water treatment plant) for immediate alteration of exposures and restoration of water resource uses. In-lake approaches are intended to manage exposures of cyanobacteria that produce MCs, while in-plant approaches are intended to manage exposures of MCs (as cellular and/or aqueous forms) in drinking waters. Approaches reviewed in this study were those for which there were peer-reviewed data to support interpretations of relative effectiveness, scalability, durability, and availability.

3 Source and characterization of microcystins

Microcystins are a group of hepatotoxins (i.e., affecting the liver) and tumor promoters that can be produced by many genera of cyanobacteria (commonly referred to as blue-green algae) including *Microcystis*, *Dolichospermum*, *Pseudanabaena*, and *Woronichinia*. To better understand microcystin production and characterize risks in our freshwater resources, it is necessary to understand characteristics of the organisms that produce those compounds. Cyanobacteria are photosynthetic, prokaryotic (single-celled) microorganisms that can grow in a range of aquatic systems including ponds, lakes, reservoirs, streams, rivers, canals, and estuaries. Cyanobacteria were the first photosynthetic organisms on Earth, with their presence dating back to the Archaean Era (2.5-3.8 billion years ago). Given this information, it is not surprising that these organisms have unique and competitive physiological traits including the ability to regulate their buoyancy in the water column to access sunlight and nutrients, higher salinity and water temperature tolerances than algae, acclimation responses to nutrient-limited conditions, and the ability to take up excess nutrients (namely phosphorus), known as luxury consumption. Cyanobacteria are naturally present in most bodies of water at relatively low densities and are a normal part of aquatic ecosystems. However, when cyanobacteria grow to cell densities in water that become visible to the human eye, this is often referred to a “bloom”, thus giving way to the common term, harmful algal bloom (HAB). Symptoms of climate change as well as increased human population and activity appear to correlate with increased frequency, duration, and intensity of HAB events in our freshwater resources. These may include warmer water temperatures, more intense precipitation events leading to increased mass loadings of nutrients into aquatic systems from runoff, lesser duration of ice cover during winter at higher latitudes, more land being used for agriculture, and less land containing vegetation or forest that could serve as riparian zones for trapping nutrients from runoff. Within an aquatic system, microcystin-producing cyanobacteria may include planktonic (i.e., free-floating in water column) species, benthic species that form assemblages on rocks, sunken detritus, and sediments, and/or filamentous mat-forming species that may cover the sediments or float to the surface of the water column. Cyanobacteria are heterogeneously distributed in water resources, where accumulations of planktonic cells are often driven to shoreline areas due to wind and wave action, creating visible blue-green surface scums. Microcystin-producing cyanobacteria have been identified in waters of every state within the United States, and all continents except for Antarctica have reported blooms of microcystin-producing cyanobacteria in surface waters. The duration and frequency of blooms is site-specific since there are many environmental and physical factors that can influence growth and colonization of each strain of cyanobacteria. For example, duration of a suitable growing season varies with latitude. Given the inherently competitive and adaptable characteristics of cyanobacteria, paired with the environmental changes taking place that favor cyanobacterial growth, it is anticipated that blooms of microcystin (and other toxin)-producing cyanobacteria will only become more frequent (spatially and temporally), more intense, and longer-lasting in our freshwater resources.

Microcystins (the group of toxins produced by some cyanobacteria) are cyclic peptides containing 7 amino acids. Over 200 structural variants of microcystins have been identified analytically, where differences lie in the placement and types of two amino acids in the compound, as well as methylation or demethylation at specific sites. Microcystins are liver toxins and tumor promoters. These compounds are primarily recognized as liver toxins because they require active transport for uptake (i.e., they do not passively move across cell membranes), and liver cells happen to contain transporting proteins that can bind with microcystins. Similar transporting proteins have been identified in kidney cells and at the blood-brain barrier. These compounds are secondary metabolites, meaning cyanobacteria do not need to produce them to survive or grow. One hypothesis is that microcystins (and other cyanobacterial toxins) may be produced as a competitive defense mechanism to ward off herbivores.

Since microcystins are produced within cyanobacteria cells, there are two phases (or forms) to be aware of, including cellular and aqueous microcystins. Cellular microcystins are defined as the fraction inside of cells, whereas aqueous microcystins are dissolved in the water. “Total microcystins” refers to the sum of cellular and aqueous concentrations. Microcystins are primarily contained within cells and are released from cells into the water upon cell death. In an actively growing bloom, there is continuous cell reproduction and death, thus there will always be some level of aqueous microcystin. When a dense bloom senesces all at once (e.g. due to change in weather or at the end of a growing season), there is likely to be a surge in aqueous microcystin.

When in the aqueous phase, sorption, photolysis and biodegradation (both aerobic and anaerobic) are dominant fate processes for microcystins. Aerobic and anaerobic biodegradation are likely to occur at a faster rate (i.e., half-lives on the order of hours to days) than photolysis in waters with relatively high total suspended solids concentrations and/or deep water columns, due to the lack of UV penetration as a result of those characteristics. Fate via drinking water treatment processes is discussed in a subsequent section.

Many countries that have recommended guidelines for microcystins in drinking water have adopted the guideline originally developed by the World Health Organization (WHO), of 1 µg per L as total (sum of cellular and aqueous) microcystins. Other methods have included the use of the tolerable daily intake derived from the peer-reviewed study that was used to establish the WHO's guideline, but differences in assumptions for body weight and/or daily water intake were used, as in the United States' guidelines for young children and adults of 0.3 and 1.6 µg per L, respectively. Some guidelines are specific to a commonly studied congener of microcystin, microcystin-LR, while others may apply to any congener of microcystins. The key take home message here is that guidelines are not federally promulgated laws. This means that by federal law, public water systems and managers of public lakes used for recreation are not required to monitor for or act on levels of total microcystins exceeding a guideline. Further, a survey provided by the Association of State Drinking Water Administrations (ASDWA) found that the majority of the states in the United States have no plans in place for detections of cyanobacterial toxins, nor are there intentions to act on the health advisory guidelines by this majority. Given the apparent increase in frequency, magnitude, and duration of microcystin-producing cyanobacteria blooms in the United States and worldwide, this could mean that millions of people and animals are getting exposed to toxins without awareness or protection.

3.1 Source of microcystin production

MCs are secondary metabolites produced intermittently or consistently by several genera of cyanobacteria (e.g. *Anabaena, Anabaenopsis, Aphanizomenon, Aphanocapsa, Coelosphaerium, Cylindrospermopsis, Gloeotrichia, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Phormidium, Planktothrix, Pseudanabaena, Synechococcus, Synechocystis,* and *Woronichinia*) in freshwater systems (Graham et al., 2004; Paerl and Otten, 2013; Paerl, 2014). Cyanobacteria are relatively competitive species (compared to eukaryotic algae) due to physiological characteristics allowing growth in a range of light and nutrient conditions (Graham and Wilcox, 2000). For example, cyanobacteria can regulate their buoyancy in water (Humphries and Lyne, 1988; Chorus et al., 2000; Zurawell et al., 2005), which allows cells to alter their depth in the water column and access nutrients, as well as the ability to move closer to light (Chorus et al., 2000). This is readily apparent in blooms formed at the water surface, which can often shade light from non-buoyant phytoplankton.

Further, several species are nitrogen fixers (Paerl et al., 2001; Paerl and Otten, 2013) and in general, cyanobacteria are capable of luxury consumption of phosphorus (taking up concentrations well in excess of environmental requirements) (Paerl et al., 2001). Cyanobacteria have unique acclimation responses to nutrient-limited conditions that include growth arrest (i.e., increased catabolism and decreased anabolism), degradation of intracellular membranes, and degradation of pigments, which can result in apparent chlorosis while cells enter a dormant stage (Schwarz and Forchhammer, 2005). However, this process can be reversed, and cells can return to a vegetative growth phase within several days following supply of adequate nutrients (Schwarz and Forchhammer, 2005). Adaptation of cyanobacteria to nutrient deprivation and their ability to recover rapidly is not surprising, since these organisms have prospered on Earth for approximately 3.5 billion years (Paerl and Otten, 2013). Thus, the concept of nutrient management for risk intervention of MCs is fundamentally flawed, which is discussed in subsequent sections of this paper. Cyanobacteria have higher salinity tolerances (i.e., limits for growth) compared to eukaryotic organisms (0 to 14.6 ppt for *Anabaena torulosa* (Apte et al., 1987)), 0 to 30 ppt for *Oscillatoria* (Fogg et al., 1973; Bishop and Premakumar, 1992), and 5 to 30 ppt for *Nodularia* (Jones et al., 1994). It is clear that cyanobacteria are competitive and opportunistic species, with wide ranges of environmental tolerances and requirements. Growth rates and occurrences of MC-producing cyanobacteria have strongly correlated with water temperatures of at least 15 ˚C and adequate exposure to sunlight, but this does not mean growth is not possible in lower water temperatures or in benthic areas with less light penetration. Efforts to monitor for and detect the presence and growth of cyanobacteria can support early warning plans for water resources given that those data are used to make an intervention decision.

Some strains of known MC-producing genera may never produce MCs due to inactive genotypes (Via-Ordorika et al., 2004), and both MC-producing and non-MC producing cyanobacteria can co-exist in water resources (Wood et al., 2012). Often, the majority of cells in a bloom are not producing endotoxins (Paerl and Otten, 2013), so triggering risk management based on cell density of cyanobacteria alone is misguided and can result in false positives. Iron deficiency has been correlated with increased MC quota (MC production in cells) (Sevilla et al., 2008) and prior batch culture experiments revealed that MC production is positively correlated with nitrogen and phosphorus concentrations (Sivonen, 1990; Vezie et al., 2002), but nutrient concentrations are more likely to be correlated with population growth rates rather than with MC quota (Sevilla et al., 2010; Neilan et al., 2012). In a laboratory experiment, MC quota increased when cells were in exponential growth phase, as compared to stationary or lag phases (Watanabe et al., 1989). Similarly, in mesocosm and lake-scale studies, Wood et al. (2010b and 2012) observed marked increases in MC quotas with increases in cell density. There are likely several environmental factors influencing MC production at any site or any point in time (Paerl and Otten, 2013). Therefore, measurements of cell density of putative MC-producing cyanobacteria alone are insufficient for triggering risk management decisions for MC exposures. However, the presence of known MC-producing cyanobacteria indicates the potential for MC production and provides evidence regarding where management efforts should be focused if MC production is occurring.

3.2 Structures and compositions of microcystins

MCs are cyclic heptapeptides with molecular weights ranging from approximately 800 to 1000 Da (Botes et al. 1982; Carmichael et al., 1988; Namikoshi et al., 1990). They are hepatotoxic (Carmichael, 1994; Feurstein et al., 2009) and phytotoxic (Bittencourt-Oliveira et al., 2014; Corbel et al., 2014), and consist of over 200 congeners (structural variants). MCs contain the general structure cyclo-(D-alanine^{1}-X^{2}-D-MeAsp^{3}-Z^{4}-Adda^{5}-D-glutamate^{6}-Mdha^{7}), where X and Z (in the 2nd and 4th positions) are variable amino acids, D-MeAsp is D-erythro-β-methylaspartic acid and Mdha is *N*-methyldehydroalanine (Hitzfeld et al., 2000). MC congeners are named by substitutions of the variable amino acids. For example, MC-LR contains leucine and arginine in positions 2 and 4, respectively. Other common congeners include MC-RR (arginine, arginine), MC-YR (tyrosine, arginine), and MC-LA (leucine, alanine). MC congeners can also differ by methylation or demethylation at specific sites in the peptide (Duy et al., 2000; Zurawell et al., 2005).

3.3 Mechanisms of toxicity of microcystins

MCs are considered hepatotoxins because a well-studied mechanism of action occurs in liver cells. Specific organic anionic transporting polypeptides (OATPs) actively transport MCs across cell membranes of hepatocytes (Carmichael, 1994). Once inside the cell, the hydrophilic Adda amino acid binds with protein phosphatases 1 and 2A, inhibiting their activity, resulting in excess phosphorylation of proteins, and ultimately, dissociation and net loss of protein filaments (Carmichael, 1994). This alteration of protein filaments causes hepatocytes to shrink, followed by a loss of adhesion between cells, breakdown of the sinusoidal structure, and pooling of blood (Carmichael, 1994; Falconer and Yeung, 1992). Although protein phosphatases and kinases exist throughout biotic systems, specific OATPs that actively transport MCs across cell membranes (due to a relatively high affinity for MCs) in mammals are found in liver and kidney cells (Feurstein et al., 2009; Campos and Vasconcelos, 2010). OATPs capable of transporting MCs are also found in the blood-brain barrier (Fischer et al., 2005; Feurstein et al., 2009), but evidence for neurotoxicity is limited for MCs. Protein phosphatases 1 and 2A are found in plants as well, and prior studies have found similar inhibition in plant cells, which is likely responsible for phytotoxic properties of MCs (MacKintosh et al., 1990).

Protein kinases and phosphatases also play roles in regulating cell division (Carmichael, 1994). Protein kinases support movement of cells through the process of cell division, while phosphatases decrease activity of regulators (Carmichael, 1994). If the function of phosphatases is decreased in this case, then cell proliferation could increase. There is no evidence that MCs are inherently carcinogenic; rather, MCs can promote growth of abnormal cells at an increased rate (i.e., tumor promoters), especially in the presence of known carcinogens (i.e., tumor initiators; Falconer, 1991; Nishiwaki-Matsushima et al., 1992; Wangth and Zhuth, 1996; Sekijima et al., 1999). Herfindal and Selheim (2006) observed that non-lethal doses of MCs in laboratory mice resulted in liver cell proliferation. This is the primary concern for humans and other organisms routinely consuming sub-lethal concentrations of MCs over the course of a lifetime (Bell and Codd, 1994; Chorus et al., 2000).

3.4 Spatial distribution of microcystin-producing cyanobacteria

All continents except for Antarctica have reported blooms of MC-producing cyanobacteria in surface waters (Carmichael, 1992; Fristachi et al., 2008), ranging from estuarine (Paerl, 1988; Robson and Hamilton, 2004; Lehman et al., 2005) to freshwater (Paerl and Otten, 2013) systems. The focus of this study is on fresh surface waters, since freshwater constitutes less than 1 percent of water globally, and majority of freshwater used by humans is from surface waters (e.g., 75% of freshwater used for drinking, irrigation, industry, agriculture, aquaculture in 2010 in the United States came from surface waters; Maupin et al., 2014). In the USEPA's National Lake Assessment of 2007, 1,161 lakes (45% natural; 55% man-made reservoirs) were sampled 1 or 2 times between May and October for MC measurements. MCs were measured in 32% of samples overall, with the majority of detections in the upper Midwestern plains and the Great Lakes (Loftin et al., 2016). In the 2012 National Lake Assessment, MC detections increased from 32% to 39% of samples overall (USEPA, 2016).

Cyanobacteria are heterogeneously distributed in water resources and can grow in slow-moving rivers, lakes, ponds, and reservoirs (Codd et al., 1999a). Accumulations of cells can be driven to shore from wind or boat action where dense blooms form (Chorus et al., 2000). Although surficial scums of planktonic species are frequently targeted as MC-producers, benthic MC-producers (e.g., *Oscillatoria* and *Phormidium*) are also prevalent and have been associated with animal poisonings (Izaguierre et al., 2007; Quiblier et al., 2013). Thus, targeted monitoring and sampling restricted to the surface of the water column could result in false negatives (e.g., conclusions that there are no MC-producing cyanobacteria present in a water resource). When cyanobacteria blooms coincide with areas of water resources with designated uses, there is potential for exposures. In regions with an abundance of freshwater resources (e.g., the Midwestern and Southeastern United States), withdrawing water from an alternative water resource may be possible if a cyanobacteria bloom occurs. However, in more arid regions (e.g., the Western and Southwestern United States), alternative water resources are likely not available. Further, as growth and colonization of MC-producing cyanobacteria increase with time and space, water resources in which these cyanobacteria have not been detected will become fewer and using alternate water resources will no longer be a viable option.

3.5 Temporal aspects for microcystin-producing cyanobacteria

The duration and frequency of MC-producing cyanobacteria blooms are site specific, since environmental and physical factors influence growth and colonization. Water temperature and light can correlate with early growth, whereas physical conditions, including minimal vertical or horizontal mixing, can sustain growth (Paerl, 2014). The duration of a growing season that can support cyanobacteria growth varies with climate and latitude; for example, blooms can occur year-round in humid, warm areas such as Florida, but only during summer months for areas farther from the equator (e.g., Minnesota). At sites with distinct growing seasons, there is often an initial rapid growth phase, followed by consistent growth and senescence of populations (i.e., continuous turnover), and finally rapid and wide-spread senescence (e.g., apoptosis), resulting in release of cellular MCs into the water column (Paerl and Otten, 2013) when environmental tolerances and requirements are no longer met by ambient conditions. Within growing seasons, blooms can also senesce suddenly following extreme weather events including flooding and droughts. Since apoptosis is inevitable and timing can be unpredictable, there are flaws in the common notion that allowing blooms to grow and persist (so long as MCs remain in cells) is a decision of lesser risk. From an exposure perspective, it is advantageous to intervene (and to be ready to intervene) early, since MC concentrations could correlate with cell density at a given site if constant MC production is occurring (Graham et al., 2008).

3.6 Fate properties of microcystins

Assuming MCs are in the aqueous phase, duration of MC exposures is a function of various fate properties for MCs (Table 1), which would be relevant following release of cell-bound MCs into water (Codd et al., 1999a). MC-LR has been widely studied as a model for MC fate properties, likely due to its common presence among other congeners (Yen et al., 2006; Dyble et al., 2008; Graham et al., 2010), relatively high toxicity among congeners (Funari and Testai, 2008), and commercial availability of analytical standards for MC-LR. Relative hydrophobicity is a fundamental property of MCs for predicting environmental fate (Liang et al., 2011). Among the few congeners for which there are hydrophobicity data, MC-RR and MC-LR are considered similarly hydrophilic at environmentally relevant pH values (6 to 8.5) (Lawton et al., 2003, Liang et al., 2011), whereas MC-LF and MC-LW are more hydrophobic (Lawton et al., 2003; Vesterkvist

and Meriluoto, 2003). Relative hydrophobicities of MC-LF and MC-LW have been estimated via indirect measurements (i.e., percent adsorption to a surface; Lawton et al., 2003) and data are lacking for comparable sorption coefficients among congeners.

MC-LR is resistant to chemical hydrolysis at near neutral pH (Table 1). Aerobic and anaerobic biodegradation half-lives are on the order of days given that bacterial MC-degraders are present (Cousins et al., 1996; Holst et al., 2003; Edwards et al., 2008; Chen et al., 2010a; Corbel et al., 2014; Iwinski et al., 2017; Kinley et al., 2018a), whereas photolysis half-lives are on the order of hours to days, and photocatalysis half-lives can occur in minutes to hours (Shepard et al., 1998; Lawton et al., 1999; Feitz et al., 1999; Lawton et al., 2003; Kinley et al. 2018b). The fate of MCs following exposure to copper-based algaecides (a management approach targeting MC-producing cyanobacteria) is a common concern expressed in peer-reviewed literature due to earlier studies (Jones and Orr, 1994) that concluded MCs will persist for weeks following algaecide applications due to biocidal effects on MC-degrading bacteria. More recent studies have shown that within legal ranges of algaecide exposure concentrations (0.1 to 1 mg Cu/L), MC biodegradation half-lives were less than 3 days for aerobic conditions (Iwinski et al., 2016a; Iwinski et al., 2017) and at dissolved oxygen concentrations of less than 2 mg per L (Kinley et al., 2018a).

3.7 Guidelines for safe levels of microcystins in the United States and globally

Several countries with drinking water guidelines or standards for MCs base their final concentration limits on the World Health Organization's (WHO) provisional guideline value of 1 µg per L (e.g., Brazil, the Czech Republic, France, Spain, and Uruguay; Ibelings et al., 2014). In some other countries, the underlying tolerable daily intake (TDI) of 0.04 µg MC per kg of body weight (derived from Fawell et al., 1994) is used, but differences in assumptions for body weight and/or daily water intake modify the final guideline value concentration slightly. For example, the USEPA defines guideline values for children (from birth to less than 6 years old) and adults (6 years old through adult) that range from 0.3 to 1.6 µg per L (USEPA, 2015). Some guidelines or standards are intended specifically for MC-LR (e.g., in Canada, the Czech Republic, and Singapore), while others are expressed in terms of total MCs (as MC-LR equivalents based on analytical method) (e.g., Australia, France, and Finland; Ibelings et al., 2014).

In regards to recreational guidelines, the USEPA recently published a recommended value of 8 µg per L for microcystins, in order to be protective for primary contact recreation (USEPA, 2019). If this value is to be used for a swimming advisory, the USEPA recommends the value not be exceeded on any given day. **It is important to recognize that health advisory guidelines in the US are not federally promulgated, thus the majority of public water systems and managers of public lakes used for recreation are not required to monitor for MC-producing cyanobacteria or MCs in raw or treated waters unless required by the state (e.g., as in Ohio).** In a survey conducted by the Association of State Drinking Water Administrations (ASDWA), the majority of states in the United States have no plan in place for detection of toxins produced by cyanobacteria, nor are there intentions to act on the USEPA health advisories since they are not regulations (AWWA, 2015), which raises significant concerns regarding unreported and unmanaged exposures in drinking waters in the United States.

In summary, components of MC exposures including sources, chemical structures, environmental fate and toxicological properties, and spatial and temporal properties (for both MCs and MC producers) are integral to understanding how to manage risks for each site-specific situation. MC-producing cyanobacteria are competitive and ubiquitous species capable of growing in a wide range of environmental conditions, thus the majority of freshwater resources could be favorable for their growth. MC-producing cyanobacteria are globally distributed, and when blooms coincide with areas of critical water resources that provide services, there is potential for exposures to occur. Given the preceding information, data regarding effects as a consequence of exposures were reviewed, vetted, organized, and assembled in the following section.

Table 1. Physical, chemical, and biological characteristics of MC-LR

Microcystin-LR (L – leucine, R – arginine)	
Formula **Molecular Weight**	$C_{49}H_{74}N_{10}O_{12}$ 995.17 g mol^{-1}
CAS Number	101043-37-2
Color/ Form	White Solid
Density	1.29 g/cm^{3}[a]
Photolysis (half-life)	30-min to 10-d[b]
Photocatalysis (half-life)	2-138-min[c]
Hydrolysis	Negligible[a]
Microbial Aerobic Degradation (half-life)	1-14-d[d]
Microbial Anaerobic Degradation (half-life)	1-3-d[e]
Solubility in Water	>1g/L[f]
Boiling point (°C)	300-341°C[f]
Log_{Dow} (n-octanol/water distribution ratio)	At pH 2 ~1.5; At pH 5 ~ -1.0; At pH >6 ~ -1.3 to -1.5[g] At pH 1 = 2.18; At pH 10 = -1.76[h]
K_d (L kg^{-1}) (Sediment)	5-35[i], 0.23-6.95[j], ~1-13[k]

[a] USEPA, 2015; [b] Tsuji et al., 1994; Tsuji et al. 1995; Kinley et al. 2018b; [c] Shepard et al., 1998; Lawton et al., 1999; Feitz et al., 1999; Shepard et al., 2002; Kinley et al. 2018b; [d] Cousins et al., 1996; Edwards et al., 2008; Iwinski et al., 2017; [e] Holst et al. 2003; Chen et al. 2010a; Kinley et al. 2018a; [f] USEPA, 2015; [g] Liang et al. 2011; [h] de Maagd et al. 1999; [i] Wu et al. 2011; [j] Miller et al. 2001; [k] Calculated from Munusamy et al. 2012 (Fig. 2, pg. 2395)

4 Human exposure routes and effects for microcystins

In order to evaluate potential risks to people from microcystin-producing cyanobacteria in our freshwater resources, the first step needs to be a thorough analysis of the potential ways that people can get exposed (i.e., come in contact with microcystins). Human exposure routes for microcystins can be categorized as direct and indirect, where direct routes include exposure directly to microcystins through various forms and indirect routes include those in which microcystins enter the body by eating food or supplements that have been contaminated from other sources.

Potential direct exposure routes include ingestion (e.g. of contaminated drinking water or of small quantities of water accidentally during swimming), inhalation (e.g. of steam during showering or of aerosolized cyanobacteria during recreation), dermal contact (e.g. during swimming or bathing), and intravenous exposure due to medical treatments. Potential indirect exposure routes include ingestion of microcystin-laden food (e.g. vegetables, fruits, grains, fish, and shellfish) and health supplements containing microcystin-producing cyanobacteria or their extracts.

A strategic literature review was conducted to identify peer-reviewed studies that met data acceptability criteria and reported measured concentrations from various exposure sources (e.g. drinking water, vegetables, blue-green algal supplements). Using data provided by the USEPA from the Exposure Factors Handbook, averages of how much of each exposure source people take in daily (e.g. average amount of water a person drinks in a day, average amount of vegetables or fruit a person eats in a day) were used along with reported microcystin concentrations to calculate an estimated daily exposure for each source. The purpose of this exercise was to assign a relative ranking among exposure routes and sources to identify where potential for risk may be greater and provide emphasis on where future risk assessments should be prioritized.

The exposure routes and associated sources for which there were sufficient and acceptable data in the literature included 1) ingestion of: drinking water, water during swimming, lettuce, carrots, tomatoes, rice, finfish, shellfish, and blue-green algal supplements, and 2) inhalation: of aerosolized cyanobacteria cells during recreation in or on water. Based on available data and calculations made, all but ingestion of rice and inhalation during recreation could result in exposures exceeding the tolerable daily intake recommended by the World Health Organization (WHO). Exposure routes that could result in the highest concentrations of microcystins included incidental ingestion of microcystins during swimming, consumption of vegetables irrigated with contaminated water, and consumption of blue-green algal supplements (e.g. potential exposures 3-6 orders of magnitude greater than recommended safe daily intake). Ingestion of drinking water, fish, and shellfish could result in relatively lower, yet relevant exposures. Based on these results, the assumptions made to develop current guidelines from the WHO and USEPA could drastically be underestimating the exposures received from sources other than drinking water. It is important to acknowledge that the calculations presented in this section are conservative and are merely intended to provide evidence of where exposures are more probable. Further, for us to conclude actual risk from these exposure routes and sources, there needs to be evidence of a complete exposure pathway. This means that there must be scientific data supporting that a given exposure of microcystins results in a measurable dose in the body, and that dose produces measurable (and relevant) adverse effects. If we do not have these data, we are limited in our ability to reliably predict risks from microcystin exposures.

A strategic literature review was conducted to identify toxicological studies focused on evaluating exposure-response relationships for microcystins and mammals, to discern if there are defensible data providing evidence of complete exposure pathways from the various potential exposure routes. Based on this literature search, there were few studies available to provide some preliminary evidence of adverse effects measured after oral exposures of microcystins in water and inhaled exposures of aerosolized microcystins in rodents. No studies were found regarding toxicity after food containing microcystins was ingested. Limited or lack of peer-reviewed data available to accurately predict risks from microcystin exposures highlights the need for more defensible data to support our risk assessments. Anecdotal evidence of human symptoms after suspected microcystin exposures includes vomiting, diarrhea, nausea, skin rashes, eye irritation, ear irritation, as well as asthmatic symptoms and nasal irritation.

Overall, the data and information presented in this section provide a case for increased attention and awareness on exposure routes in addition to ingestion via drinking water. For example, the possibility for exposures via ingestion of contaminated crops is of concern, given that microcystin concentrations are not regulated in irrigation waters or in food

sources. Clearly, human health risks related to food-borne exposures of MCs warrant just as much (if not more) attention and scientific investigation, similar to risk assessments conducted for drinking water and recreational exposures.

4.1 Human exposure routes

Exposures of MCs have characteristics of concentration, duration, frequency, form, and route. In this context, concentration, duration, form, and frequency are specific to the route of exposure. Direct MC exposure routes for humans may include ingestion of drinking water, incidental ingestion of surface waters during swimming and recreation, inhalation of steam during showering, inhalation of aerosols during recreation, dermal contact during swimming or bathing, and intravenous exposure during medical treatments (Codd et al., 1999a; Carmichael, 2001; Funari and Testai, 2008) (Figure 2). Indirect exposure routes include ingestion of MC-laden food (e.g., vegetables, fruits, grains, fish, and shellfish) and health supplements containing cyanobacteria or extracts of cyanobacteria (Codd et al., 1999a; Carmichael, 2001; Funari and Testai, 2008) (Figure 2).

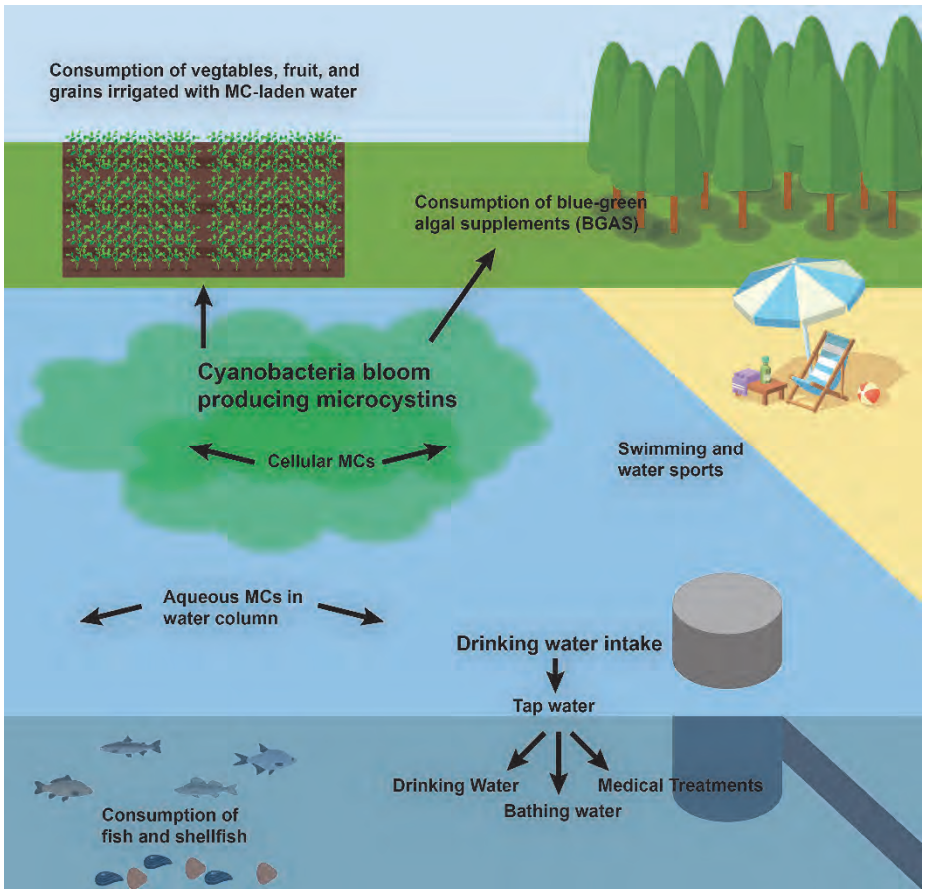

Figure 2. Conceptual model of potential human exposure routes for microcystins

4.1.1 Ingestion of drinking water

In drinking waters, exposures are a function of the total MC concentration and form (cellular or aqueous) in source waters, the periodicity of a bloom in source water, the proximity of the MC-producing bloom to the drinking water intake, and the effectiveness of in-lake (i.e., source water) and in-plant (i.e., drinking water treatment) mitigation approaches. Measured (and reported) exposure concentrations in treated drinking waters have ranged from 0.1 to 12.5 µg per L globally (Lahti et al., 2001; Blaha and Marsalek, 2003; Hoeger et al., 2005; Jacoby and Kann, 2007; Burns, 2008), whereas the majority of available data show non-detectable MC concentrations. Based on average daily drinking water ingestion (USEPA, 2011) and the measured range of MC concentrations in drinking waters, potential daily exposures could range from 0.004 to 4.5 µg MC for infants through 5 years of age, and 0.01 to 13.1 µg MC for people older than 6 years, including adults (Table 2). Again, in the majority of situations, MC concentrations in final treated

drinking waters are non-detectable (i.e., less than 0.1 µg per L), but the purpose for this calculation is to estimate potential exposures for humans that could occur from MC concentrations that have been measured in drinking waters.

4.1.2 Incidental ingestion of water during swimming or recreation

Exposures in surface waters during recreation are a function of total MC concentrations, frequency and duration of blooms, and proximity of the bloom to individuals accessing the water resource for recreation. Total MC concentration (cellular and aqueous combined) in lakes and reservoirs can range from parts per billion to parts per million (Graham et al., 2010; Heiskary et al., 2014; Howard et al., 2017). For exposure estimations, a range of maximum measured total MC concentrations in surface waters from the peer-reviewed literature were used. MC concentrations in the United States ranged from 189 to 36,549 µg per L (Graham et al., 2010; Heiskary et al., 2014; Loftin et al., 2016; Howard et al., 2017). Children (e.g., less than 17 years old) and adults (more than 17 years old) consume an average of 49 and 21 mL of water per hour, respectively, when swimming (Dufour et al., 2017), so possible exposures based on maximum measured MC concentrations could range from 9.3 to 1,790 µg for children and 4 to 767.5 µg for adults for one hour of swimming (Table 2).

4.1.3 Ingestion of vegetables and fruit

MC exposures in vegetables and fruits are a function of periodicity (e.g., amplitude, frequency, and duration) of blooms, the initial exposure concentration in irrigation water, and water uptake rates by plants. Based on primary evidence, MC concentrations in lettuce leaves have ranged from 5 to 178 µg per kg fresh weight (fw) following irrigation with MC-laden water containing concentrations from 1 to 13 µg per L MC (data in supplementary material; Table S1). Similarly, MC concentrations in carrots ranged from 10 to 200 µg per kg fw (Table S1). Green and ripe tomatoes contained approximately 5 to 11 µg MC per kg fw following irrigation with water containing 100 µg MC per L (Table S1). Secondary evidence included measured MCs (without knowledge of initial exposure) in lettuce and arugula leaves of 4.7 to 400 µg MC per kg fw (Codd et al., 1999b; Mohamed and Shehri, 2009) and up to 1200 µg MC per kg fw in cabbage (Mohamed and Shehri, 2009). Jarvenpaa et al. (2007) measured MCs in seedlings of broccoli (*Brassica oleracea* var. *italica*) and mustard (*Sinapis alba*) following exposures to MCs extracted from laboratory cultures of *Anabaena* for 19 to 20 days. Roots of broccoli and mustard plants contained 0.9 to 2.4 and 2.5 to 2.6 µg MC per kg fw, respectively (Jarvenpaa et al., 2007). Chen et al. (2010b) measured MC-LR concentrations of approximately 16, 28, and 225 µg MC per kg fw in apple tree shoots (*Malus pumila*) following aqueous exposures of 30, 300 and 3000 µg MC per L of water for 7 days. Based on average daily intake of vegetables for infants through 5 year olds, exposures from lettuce, carrots, and tomatoes could range from 0.63 to 22.5, 1.3 to 25.3, and 0.63 to 1.4 µg MC, respectively (Table 2). For adults, exposures could range from 1.5 to 52.7, 2.9 to 59.2, and 1.5 to 3.3 µg MC, respectively, for lettuce, carrots, and tomatoes (Table 2).

4.1.4 Ingestion of grains

Similar to vegetables and fruits, MC exposures in grains are a function of periodicity of blooms, proximity of the bloom to the irrigation intake, the initial concentration of MC in irrigation water, and water uptake rates by plants. Studies found for grain exposures met criteria for secondary evidence. Chen et al. (2004) exposed rice (*Oryza sativa*) to aqueous solutions of MCs at concentrations ranging from 24 to 3000 µg per L for 10 days. Average MC concentration in these rice seedlings ranged from 2.94 µg MC per kg fw (exposed to 120 µg per L) to 5.4 µg MC per kg fw (exposed to 3000 µg per L) (Chen et al., 2004). Chen et al. (2012) measured MC-LR in rice grains collected from rice fields adjacent to Taihu Lake (China), which repeatedly experienced cyanobacterial blooms during the rice growing season (May to November), and detected MC-LR in 21 of 44 samples, with concentrations ranging from 0.04 to 3.19 µg per kg dry weight (dw) (Chen et al., 2012). Based on these limited data constituting secondary evidence, MC exposures from rice could range from 0.005 to 0.38 µg for infants and children through 6 years of age, and from 0.02 to 1.1 µg for children older than 6 through adults (Table 2).

4.1.5 Ingestion of fish

For fish exposure data, targeted studies were those in which MC concentrations were measured in fish collected from aquatic systems that contained MC-producing cyanobacteria at the time of fish collection. Among fish species including

rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), and European flounder (*Platichthys flesus*), maximum measured MC concentrations in muscle tissue ranged from 1.6 to 100 µg per kg fw (Mohamed et al., 2003; Li et al., 2004; Cazenave et al., 2005; Shen et al., 2005; Wood et al., 2006), with an outlier of 370 µg per kg fw (Chen et al., 2007) (Table S2 in supplementary material). These MC concentrations are based on methanol or methanol-butanol extractions, which routinely underestimate total MC concentration in tissues (Ibelings et al., 2005). Therefore, the reported MC concentrations may underestimate total MC concentrations in fish muscle tissue but are likely representative of the bioavailable fraction to humans (Ibelings et al., 2005; Ibelings and Chorus, 2007). Based on the average daily fish consumption by humans in the US and the range of MC concentrations measured in fish muscle tissue, possible daily MC exposures could range from 0.007 to 2.9 µg for young children and 0.014 to 5.4 µg for school-aged children through adults (Table 2). Although the mass of MCs estimated from ingestion of fish is much less than that from vegetables, in some regions, individuals may consume proportionally more fish than the average estimate, which would be an important consideration for site-specific risk assessments.

4.1.6 Ingestion of shellfish

Chen and Xie (2005a) measured MC concentrations in whole organisms for four freshwater bivalves, *Anodonta woodiania*, *Hyriopsis cumingii*, *Cristaria plicata*, and *Lamprotula leai* collected from Meiliang Bay in Lake Taihu, China. Mean MC concentrations in whole mussels (excluding intestines) calculated using data provided by Chen and Xie (2005a) and assuming 87% moisture in mussels (USFWS, 2000), ranged from 20 to 130 µg per kg fw (Table S2). Chen and Xie (2005b) measured an average of 5 µg MC per kg fw in muscle of the crayfish, *Procambarus clarkia* (calculated based on dry weight concentration and estimated 90% moisture content; Ibelings and Chorus, 2007). Additionally, Chen and Xie (2005b) reported mean MC concentrations of 6 and 4 µg per kg fw in muscle tissue of the shrimp *Palaemon modestus* and *Macrobrachium nipponensis*, respectively, and maximum muscle tissue concentrations of 26 and 12 µg MC per kg fw, respectively. Mean MC concentrations calculated in whole shrimp from data provided by Chen and Xie (2005b), assuming 90% moisture, were 97.2 and 40.1 µg per kg fw for *P. modestus* and *M. nipponensis*, respectively (Table S2). Based on the range of measured MC concentrations in shellfish and the average daily consumption of shellfish in the US, possible MC exposures could range from 0.07 to 2.4 µg for young children and 0.2 to 7.5 µg for school-aged children through adults (Table 2).

4.1.7 Ingestion of blue-green algal supplements (BGAS)

Exposures of MCs via blue-green algae supplements (BGAS) are influenced by the total MC concentrations in algal cells used to prepare supplements (resulting in MC concentrations in BGAS), dose of BGAS consumed per day, and frequency of consumption (i.e., daily, twice daily, etc.). BGAS are primarily produced from *Spirulina* and *Aphanizomenon* grown in laboratory cultures or collected directly from aquatic systems (Gilroy et al., 2000; Saker et al., 2005; Funari and Testai, 2008) and are sold as tablets, powder, and capsules. The majority of BGAS made from *Aphanizomenon* are sourced from Lake Klamath in Oregon, USA, where *Microcystis* also grows (Carmichael et al., 2000; Saker et al., 2005; Vichi et al., 2012). BGAS are often consumed in weight loss programs, as energy supplements, and are administered to children as an alternative treatment for attention deficit hyperactivity disorders (Gilroy et al., 2000; Saker et al., 2005; Dietrich and Hoeger, 2005). Measured MC concentrations in BGAS have ranged from 0.1 to 10.8 µg MC per g BGAS, with the exception of an outlier (35 µg per g; Dietrich and Hoeger, 2005). BGAS are consumed in doses of 1 to 4 g daily (Gilroy et al., 2000; Lawrence et al., 2001; Hoeger and Dietrich, 2004; Saker et al., 2005; Vichi et al., 2012). Assuming a dose of 4 g per day and the measured MC concentrations in BGAS of 0.1 to 10.8 µg MC per g BGAS, daily exposures could range from 0.4 to 43.2 µg MC (Table 2). An important consideration for BGAS is that they can be consumed year-round, which would result in continuous exposure. In water or food, typical exposures are a function of the spatial and temporal dynamics specific to a site, and are unlikely to be continuous (Chorus et al., 2000).

4.1.8 Inhalation

Inhalation of MCs may occur via steam from showering and bathing, or from aerosols during water sports (e.g., boating and jet skiing) or work-related activities involving cyanobacteria cells that produce MCs (e.g., spray water for irrigation) (Codd et al., 1999a; Carmichael, 2001; Funari and Testai, 2008; Wood and Dietrich, 2011). Cyanobacteria cells and MCs can be aerosolized when wind activity causes wave action in surface waters, ejecting cells and MCs into the air by droplets (Blanchard and Syzdek, 1972; Henegan et al., 2017). Exposures are influenced by the total MC concentration,

site characteristics influencing aerosol formation and transport (e.g., wind and wave action), and duration/frequency of inhalation by individuals. For example, humans inhabiting shoreline properties along lakes with frequent cyanobacteria blooms are likely exposed at increased frequencies and durations compared to humans not living in proximity to a water resource containing MC-producing cyanobacteria. No studies were found regarding measured MC exposures in shower or bathwater steam. In terms of aerosolized MC exposures, Backer et al. (2010) measured up to 0.052 ng per m^3 of total MCs in air, when total MC concentrations in water ranged from 14.5 to 357 µg per L in two California lakes. In a New Zealand lake containing a bloom of *Microcystis*, *Anabaena*, and *Aphanothece*, with a maximum measured total MC concentration of 2140 µg per L in the water, maximum measured MC in the air was 0.0018 ng per m^3 (Wood and Dietrich, 2011). Based on these limited data, aerosolized concentrations of MCs are relatively low compared to measured aqueous concentrations. Given the average inhalation rates by individuals (USEPA, 2011), and the range of air-bound MC exposure concentrations previously reported, possible exposures could range from 0.0008 to 0.02 ng for young children and 0.001 to 0.035 ng for school-aged children and adults during one hour of recreation (Table 2). Although MC exposures via inhalation are not anticipated to be significant for individuals engaged in occasional recreation, duration and frequency of inhalation exposures for individuals who live or work in direct proximity to water resources containing MC-producing cyanobacteria blooms are of greater concern. These types of prolonged, repeated exposures require more attention in human health risk assessments.

4.1.9 Dermal contact

Contact with cyanobacteria cells has often been associated with allergic-type reactions (e.g., skin rashes, mouth sores, eye and ear irritation; Hunter, 1998; Funari and Testai, 2008); however, direct correlations between MC concentrations and adverse effects have not been reported. Although MCs alone likely have minimal risk for uptake by skin cells, adverse responses from contact with cyanobacteria cells are considered a human health risk. Reported effects for individuals following contact with MC-producing cyanobacteria are discussed in the effects section of this paper.

4.1.10 Intravenous MC exposure via medical treatments

Hemodialysis treatment (or any other medical procedure in which water is introduced intravenously) can result in immediate contact of MCs with the bloodstream if MCs are present in waters used for treatments. Further, volumes of water used in dialysis are orders of magnitude greater than what a human would be exposed to from drinking water (e.g. up to 150 L per treatment, 3 to 4 times per week [Jochimsen et al., 1998] compared to 2 L of drinking water consumed per day [USEPA, 2015]). The hemodialysis case study in Caruaru, Brazil is the most widely reported event of human mortality following exposure to MCs (Jochimsen et al., 1998; Azevedo et al., 2002). Of 124 patients that received dialysis treatments in February 1996 (time of suspected exposure), 101 experienced acute liver injury and 50 died within 7 months (Jochimsen et al., 1998). Given the relatively higher vulnerabilities of individuals receiving these treatments and the increase in exposure concentrations that are possible as a function of the volume of water exposed per treatment (Funari and Testai, 2008), there is clearly potential for exposures via this route.

Based on the preceding data and exposure calculations, all exposure routes with the exceptions of ingestion of rice and inhalation could result in total daily intakes that exceed the TDI recommended by the WHO (Table 2). Exposure routes that could result in the greatest magnitude of exposure include incidental ingestion of MCs during swimming, consumption of vegetables irrigated with MC-laden water, and ingestion of BGAS (Table 2). Therefore, the assumption by the WHO and the USEPA that 80% of MC exposures come from drinking water (WHO, 2003; USEPA, 2015) could underestimate MC doses that may be received from food exposures. Ingestion of drinking water, fish, and shellfish could result in less exposure than ingestion of water during swimming, ingestion of vegetables, and ingestion of BGAS based on available data, but still clearly warrant additional study. For individuals receiving medical treatments that require treated drinking water, exposures of MCs are clearly relevant and significant. The probability of exposures via different exposure routes does not necessarily correlate with probability for risk unless exposure-response relationships support potential for risk. In the following section, potential effects as a consequence of exposures are reviewed.

Table 2. Estimated exposures from various exposure routes for humans based on measured MC concentrations in exposure source and daily intake of each exposure source. TDI= tolerable daily intake

Exposure source	Human age (yr)	Daily ingestion rate* (g/kg BW/d unless otherwise noted) (Maximum of mean values for age group)	Range of [MC] in source (µg/kg fw unless otherwise noted)	Estimated MC exposure (µg/d) (unless otherwise noted)	Exceed TDI? (Y/N) 0.74 µg (birth-5) 3.2 µg (6-adult)
Drinking water	Birth-5 6-adult	0.36 L/d 1.05 L/d	0.01-12.5 µg/L[a]	0.004-4.5 0.011-13.1	Y Y
Water during swimming	<17 ≥17	49 mL/h[b] 21 mL/h[b]	189-36549 µg/L[c]	9.3-1,790 4-767.5	Y Y
Lettuce*	Birth-5 6-adult	6.8 3.7	5-178[d]	0.6-22.5 1.5-52.7	Y Y
Carrots*	Birth-5 6-adult	6.8 3.7	10-200[e]	1.3-25.3 3-59.2	Y Y
Tomatoes*	Birth-5 6-adult	6.8 3.7	5-11[f]	0.63-1.4 1.5-3.3	Y Y
Rice*	Birth-5 6-adult	6.4 4.4	0.04-3.19[g]	0.005-0.38 0.01-1.1	N N
Finfish*	Birth-5 6-adult	1.6 0.68	0.25-100[h]	0.007-2.9 0.014-5.4	Y Y
Shellfish*	Birth-5 6-adult	1 0.72	4-130[i]	0.07-2.4 0.2-7.5	Y Y
Blue-green algal supplements	Birth-5 6-adult	4 g/d[j]	0.1-10.8 µg/g[j]	0.4-43.2	Y Y
Inhalation: recreation	Birth-5 6-adult	0.42 m^3/h[k] 0.68 m^3/h[k]	0.0018-0.052 $ng/m^{3(l)}$	0.0008-0.02 ng 0.001-0.035 ng	N N

* Calculations for average daily intake based on body weight (bw) of 18.6 kg for age group birth-5, and based on 80 kg bw for age group 6-adult (USEPA, 2011). Weights based on maximum value for each age group.

[a] Lahti et al. (2001); Blaha and Marsalek (2003); Hoeger et al. (2004); Jacoby and Kann (2007); Burns (2008); [b] Dufour et al. (2017); [c] Graham et al. (2010); Heiskary et al. (2014); Loftin et al. (2016); USEPA (2016); Howard et al. (2017); [d] Hereman & Bittencourt-Oliveira (2012); Bittencourt-Oliveira et al. (2016); Lee et al. (2017); [e] Lee et al. (2017); [f] Gutierrez-Praena et al. (2014); [g] Chen et al. (2012); [h] Mohamed et al. (2003); Li et al. (2004); Cazenave et al. (2005); Shen et al. (2005); Wood et al. (2006); [i] Chen and Xie (2005a and b); [j] Gilroy et al., 2000; Lawrence et al., 2001; Hoeger & Dietrich, 2004; Saker et al., 2005; Vichi et al., 2012; [k] Based on values from USEPA (2011) adjusted for 1-h (from values originally based on 24-h); [l] Backer et al. (2010); Wood and Dietrich (2011)

4.2 Effects data for humans exposed to MCs

Although MC exposures are probable from direct and indirect sources, the potential for risk is influenced by whether each exposure route can result in a complete exposure pathway, defined here as a measured exposure, resulting in a measured dose (e.g., µg MC per kg body weight), producing measurable (and relevant) adverse effects. Those data are derived from laboratory toxicity experiments with appropriate receptors for extrapolating predictions of exposure-response relationships from mammals to humans. If data were unavailable to confirm complete exposure pathways based

on the literature review and defined acceptability criteria, data gaps were identified and potential for exposures was concluded, if supporting data were available in the preceding subsection 4.1. Following evaluation of data available from laboratory toxicity experiments, reported human effects following probable exposures (i.e., anecdotal reports) were reviewed.

4.2.1 Laboratory studies regarding ingestion of microcystins

Two studies incorporated drinking water as an exposure medium for pigs and rats (Falconer et al., 1994 and Heinze, 1999, respectively). Falconer et al. (1994) reported MC concentrations in drinking water, whereas Heinze (1999) did not report measured MC exposures, and neither study reported measured doses based on body weight or in target organs (i.e., liver, kidney). Falconer et al. (1994) reported a lowest observed effect concentration (LOEC) of 280 µg MC per kg of body weight for pigs (44-day duration) based on nominal (i.e., targeted but not measured) exposure concentrations, while Heinze (1999) reported a LOEC of 50 µg MC per kg of body weight per day for mice (28-day duration), based on observed liver injury at a cellular level (via histopathological examinations) (exposure concentrations not measured). Fawell et al. (1994) reported a LOEC of 200 µg MC per kg of body weight per day for mice in 13-week gavage exposures, also based on histopathological changes in hepatocytes, as well as some changes in serum enzymes. Fawell et al. (1994) reported that exposures were measured using high performance liquid chromatography (HPLC), but did not provide exposure data, and measured doses in mice were not provided. While these studies provide strong evidence for exposure-response relationships via oral ingestion of MCs in water (given the mechanism of action of MCs and measured effects), data did not meet our criteria for confirming complete exposure pathways.

In regards to food and supplement exposures, there were no studies that measured exposure-response relationships; thus, our conclusions regarding potential for risk are based only on reported concentrations in food and supplements. To predict effects based on measured exposures in food sources or supplements, toxicity experiments would incorporate measured exposures, resulting in measured doses (and relative partitioning to organism from food or supplement source), and measured responses. The lack of studies meeting the criteria for prediction of risks to humans via ingestion highlights a significant data gap for risk management of MCs, and therefore, an opportunity to fill these data gaps. With further contributions of defensible data (i.e., meeting acceptability criteria; e.g., USEPA, 2002), uncertainty factors in regulatory standards and guidelines could be decreased, thus increasing our ability to accurately predict the potential for adverse human health effects.

4.2.2 Effects resulting from inhalation of MCs

Benson et al. (2005) exposed 6 to 8-week-old mice to 260 µg MC per m^3 (260 ng MC per L of air) for intervals of 30, 60 and 120 minutes per day for 7 days. Based on estimated aspiration rates of mice, the estimated doses were 3, 6, and 12.5 µg MC per kg of body weight per day (Benson et al., 2005). Benson et al. (2005) did not observe mortality, changes in body mass, or changes in liver mass related to MC exposure concentration, but they did observe lesions in the nasal cavities of mice exposed to 6 and 12.5 µg MC per kg of body weight per day, indicating MCs likely remained in the upper respiratory tract from those exposures. In comparison, Fitzgeorge et al. (1994) observed hepatic lesions in mice exposed to 31 µg MC per kg of body weight per day via inhalation for 7 consecutive days. It should be noted that exposure concentrations used by both Fitzgeorge et al. (1994) and Benson et al. (2005) are 7 to 8 orders of magnitude higher than concentrations that have been measured in air in immediate proximity to water resources containing MC-producing cyanobacteria (Backer et al., 2010; Wood and Dietrich, 2011). Again, it is important to consider that individuals living or working in near proximity to water resources containing MCs are likely at greater risk for chronic exposures via inhalation, even if exposure concentrations are relatively low. Therefore, chronic exposures to MCs via inhalation for select groups of individuals clearly require further scientific investigation.

4.2.3 Evidence of adverse effects in humans following suspected exposure to MCs

Reported human health effects include symptoms following suspected or known ingestion of MCs, either from drinking water or recreation. Symptoms include vomiting, diarrhea, and nausea (i.e., gastroenteritis), while other symptoms include skin rashes, eye irritation, and ear irritation (presumably from contact with cyanobacteria cells following recreation) (Bell and Codd, 1994; Pilotto et al., 1997; Cheung et al., 2013). Symptoms including asthma and nasal irritation have also been reported, likely from inhalation of cyanobacteria cells (Wood, 2016). Direct measurements of

MCs associated with these symptoms are unavailable, thus these reported symptoms are insufficient to draw causality to MCs directly. Yet, the number of case studies in which humans have reported adverse effects following contact with cyanobacteria, and the frequency at which the same types of symptoms are reported, provide strong evidence for human health risks from exposure to toxin-producing cyanobacteria.

The probability of risk is a function of exposures (i.e., concentration, frequency, duration, form, and route) and exposure (dose)-response relationships. Clearly there are data gaps regarding exposures and responses in terms of potential for risks to human health, and the process of decision making for risk management can be adapted and improved with further scientific knowledge. However, enough is known regarding probability for adverse effects from various exposure routes that water resource managers can recognize the risks associated with human exposure to MCs and the importance of risk intervention. For example, total MC concentrations should be orders of magnitude greater in surface waters used for recreation than in drinking waters during a bloom event, thus the probability of exposures resulting in adverse effects could be greater for recreational activities. Carmichael (2001) acknowledged that the probability of significant human poisonings from MCs is negligible from water, unless a relatively large quantity of cells is ingested, which would likely only be possible in recreation situations. The possibility for exposures via ingestion of contaminated crops is of concern, given that MC concentrations are not regulated in irrigation waters or in food sources (Corbel et al., 2014; Lee et al., 2017). Given the lack of attention paid to this exposure route, it is possible that adverse effects have occurred but may have been attributed to a different causative contaminant with similar effects (e.g., contamination with infectious organisms). Clearly, human health risks related to food-borne exposures of MCs warrant just as much (if not more) attention and scientific investigation, similar to risk assessments conducted for drinking water and recreational exposures. In addition, the correlation between chronic exposures to MCs and tumor promotion is still poorly understood.

5 Review of ecological toxicity data for microcystins

Toxicity to animals and plants from microcystin exposures is readily apparent and well documented, yet rarely discussed in the context of risk assessments and risk management. The goal of this section was to conduct strategic literature reviews to mine available databases for toxicological data in reference to mammals, fish, aquatic invertebrates, birds, plants, and algae exposed to microcystins. Criteria were established in order to qualify available studies as primary evidence or secondary evidence to prioritize toxicity data derived from defensible toxicity experiments over anecdotal reports or studies lacking certain quality control parameters. Data that met criteria for primary evidence were used to develop a species sensitivity distribution, which can illustrate the relative sensitivities among plants and animals and provide a focus for future risk assessments. Based on the ecological toxicity data assembled from the peer-reviewed literature, in terms of relative sensitivities, plants ≥ fish > aquatic invertebrates. Studies found for mammals and birds were either lacking necessary quality control parameters in experimental design or presented anecdotal evidence, and therefore data were not included in the sensitivity distribution. However, these data did meet criteria for secondary evidence and provided correlations to microcystin toxicity.

Adverse effects in plants were observed at exposure concentrations as low as 1 µg per L as total microcystins, and with each order of magnitude (i.e., factor of 10) increase in exposure concentration, approximately 30-40% more of the distribution of evaluated species were adversely affected. Given that environmentally relevant concentrations of microcystins can range from < 1 to thousands of µg per L in aquatic systems, there is clearly potential for ecological risk when growths of microcystin-producing cyanobacteria are present in ponds, lakes, streams, and rivers. To date, the majority of risk management efforts for microcystins have focused on drinking water and recreation for humans. The ecological toxicity data reviewed in this study clearly show that risk management is also needed for plants and animals.

5.1 Toxicity data for mammals exposed to microcystins

Mammals can be exposed to MCs via ingestion of contaminated water, shoreline deposits of cyanobacteria scums, or food items containing MCs (i.e., plants, fish). In prior laboratory studies, rats and mice have been exposed via intraperitoneal (IP) injection and gavage (force feeding) (mammalian toxicity data in supplemental material; Table S3). Apparent sensitivities have been 30 to 100 times less for mice exposed to MCs via gavage as compared to IP injection (Fawell et al., 1994), and clearly, IP injections are not environmentally relevant exposures for mammals. Based on limited available data constituting secondary evidence (none met all specified criteria for primary evidence), mice were apparently more sensitive than rats on a body mass basis when exposed to MCs via gavage for 14 days (Fawell et al., 1994; Table S3). For gavage exposures over the course of 14 days, 20% mortality was observed in male rats at 5000 µg MC per kg of body weight, and approximately 50% mortality was observed among male and female mice at 5000 MC per kg of body weight (Table S3), but complete exposure-response relationships were not measured. A complete exposure-response relationship is defined as measured responses ranging from 0 to 100% mortality, with sufficient statistical resolution to predict specific thresholds (e.g., LC_{25} or LC_{50}). For gavage exposures over the course of 13 weeks, no mortality was noted in mice treated with up to 1000 µg MC per kg of body weight, but several sub-lethal effects were observed in liver cells (Fawell et al., 1994). Potency of MC exposures for mice via the oral route is relatively low. For example, an increase from 1580 to 5000 µg MC per kg of body weight was necessary to elicit a change from 10% mortality to 50% mortality (Fawell et al., 1994).

Additional secondary evidence is available in the form of numerous post-hoc reports of livestock (e.g., cow and sheep) mortality (Galey et al., 1987; Van Halderen et al., 1995; Mez et al., 1997; Puschner et al., 1998; Frazier et al., 1998) and dog mortality (Wood et al., 2010a; van der Merwe et al., 2012; Lurling and Faassen, 2013) following ingestion of cyanobacteria scums in surface waters. In these case studies, livestock exhibited signs of recumbency, weakness, anorexia, and bloody diarrhea (Fitzgerald and Poppenga, 1993; Haynie et al., 2013). Following necropsies of deceased animals, veterinarians observed severe liver hemorrhaging, necrosis, edema, and lesions (Van Halderen et al., 1995; Mez et al., 1997), which align with anticipated effects of exposure to hepatotoxins. Domestic pets and livestock have anthropogenic value, either due to enhancement of well-being as companions, or from contributions to agricultural productivity. Mortalities of these animals (from ingestion of MCs or MC-producing cyanobacteria) resulting in significant personal and financial losses are likely to continue in situations where exposures are not managed effectively. Overall, potency of MCs is relatively low for mammals (in terms of increase in response with incremental increase in exposure), but poisonings and mortalities are still frequent, likely because total MC concentrations in shoreline scums

of cyanobacteria can reach mg per L levels, and often livestock do not have an alternate water resource or do not avoid drinking cyanobacteria in water.

5.2 Toxicity data for fish exposed to microcystins

Fish can be exposed to MCs via direct contact or ingestion of algal cells or MC-tainted food sources. Passive uptake of MCs across gill membranes is not anticipated given the structure of MCs, but ingestion of MCs or cyanobacteria cells is likely. For loaches (*Misgurnus mizolepis*), 7-day LC_{50} values occurred at approximately 164 and 593 µg MC per L for larvae and juveniles, respectively (Liu et al., 2002; Table S4). In 72-hour exposures, the LC_{50} for direct contact to tetras (*Astyanax bimaculatus*) was approximately 243 µg MC per L, while the LD_{50} for IP injection was an order of magnitude less at 49 µg MC per L (Silva et al., 2010). In general, LC_{50} values ranged from 164.3 to 593 µg MC per L for larval through juvenile fish (Figure 3; Table S4). Zebrafish embryos (*Danio rerio*) were apparently more sensitive, as exposure to an aqueous concentration of 5 µg MC per L resulted in 60% mortality (Figure 3; Table S4). Potency slopes are relatively steeper for exposure-response relationships in fish as compared to mammals. For example, Liu et al. (2002) observed a change in mortality of newly hatched loach larvae from 20% to 50% with an increase in exposure concentration from approximately 125 to 150 µg MC per L, and 100% mortality at a concentration of approximately 350 µg MC per L for a 7-day exposure duration. Effects thresholds following MC exposures via IP injection are an order of magnitude lower than effects thresholds following exposures via direct contact. Since IP injections are not environmentally relevant routes of exposure for fish, only data pertaining to aqueous exposures were included in the SSD (Figure 3).

5.3 Toxicity data for aquatic invertebrates exposed to microcystins

Aquatic invertebrates can be exposed to MCs via aqueous exposures or ingestion of algal cells containing MCs. For *Diaptomus birgei*, 24-hour and 48-hour LC_{50} values were 980 and 450 µg MC per L, respectively (DeMott et al., 1991; Table S5) in direct contact exposures. Sensitivities of three *Daphnia* species were ranked as *D. pulex* > *D. hyalina* > *D. pulicaria*, and LC_{50} values for these daphnids were 1 to 2 orders of magnitude greater than the LC_{50} for *D. birgei* (DeMott et al., 1991; Figure 3). Chen et al. (2005) measured 24-hour and 48-hour LC_{50}s for *D. magna* of 47,000 and 20,000 µg MC per L, respectively, which were similar to those measured for *D. pulicaria* by DeMott et al. (1991). When exposed to MCs for relatively longer durations of 21 days in the same study, the LOEC for *D. magna* (~17% mortality) decreased to 640 µg MC per L, and the LOEC for reproduction was 360 µg MC per L (Chen et al., 2005). An LC_{50} and EC_{50} could not be calculated for the 21-day experiments conducted by Chen et al. (2005) since a complete exposure-response relationship was not achieved. However, for exposure concentrations of 1000 and 2000 µg MC-LR per L, 50% mortality of *D. magna* was observed (Chen et al., 2005). These data highlight the significance of considering duration of exposures when interpreting toxicity data within and among species, since magnitude alone is not the sole influence on exposures and consequent responses (Klaassen, 2008). For example, from 24-hour to 48-hour exposures, LC_{50} values for *D. magna* decreased by more than half (47,000 vs. 20,000 µg MC per L) and further decreased to approximately 1000 µg MC per L after 21 days (Chen et al., 2005). *Daphnia* spp. are less sensitive than fish previously evaluated, given that 7-day LC_{50} values for larval and juvenile loaches (*M. mizolepis*) were greater than 1000 µg MC per L (Liu et al., 2002) and a 30-day LOEC for growth of 70-day-old zebrafish (*D. rerio*) was approximately 1 µg MC per L (Liu et al., 2014).

5.4 Toxicity data for birds exposed to microcystins

Birds can be exposed to MCs by incidental ingestion of cyanobacterial cells from surficial scums during drinking of water, through ingestion of food containing MCs, and through ingestion of contaminated water from preening of feathers within aquatic systems impacted by MC-producing cyanobacteria (Krienitz et al., 2003). Evidence of avian exposures to MCs is in the form of observations of bird mortalities and correlations with algal bloom events (Matsunaga et al., 1999; Carmichael and Li, 2006; Papadimitriou et al., 2018). For example, death of 91 Dalmatian pelicans (*Pelecanus crispus*) in the Karla Reservoir (Greece) prompted measurements of cyanobacterial toxins (MCs, cylindrospermopsins [CYNs], and saxitoxins [STXs]) in the reservoir water, cyanobacterial scums, and in dead pelican tissues (Papadimitriou et al., 2018). The livers of dead pelicans contained average MC concentrations of 231.3 µg per kg of liver weight and average CYN concentrations of 148.3 µg per kg of liver weight (Papadimitriou et al., 2018). In a similar event, observations of mortalities of about 20 spot-billed ducks (*Anas zonorhyncha*) in a pond in Nishnomiya, Hyogo Prefecture, Japan (September 1995) prompted measurements of MCs in the pond and in an adjacent pond, since floating

cyanobacterial scums were also observed in the water (Matsunaga et al., 1999). MC concentrations of approximately 318, 33, and 161 µg per g dw of cyanobacteria cells of MC-RR, MC-YR, and MC-LR, respectively, were measured in the visible scum.

Two studies were conducted to expose young Japanese quail (*Coturnix japonica*) to known concentrations of MCs from cyanobacterial extracts, yet neither experiment yielded measurable adverse effects in birds (Skocovska et al., 2007, Damkova et al., 2009). For example, Skocovska et al. (2007) exposed 4-month-old quail (average 205 g body weight) to a range of MC concentrations (extracted from an assemblage of *Microcystis* species) that spanned 3 orders of magnitude via dietary exposure for operationally defined acute (10-day) and sub-chronic (30-day) durations. Using crop probes, birds were force-fed 10 mL of MC masses three times daily (n=5 males for each exposure). Each mass contained 0.045, 0.46, 4.6, or 46.04 µg MC, which based on the average mass of the experimental quail, is comparable to 0.2, 2.2, 22, and 222 µg MC per kg of body weight (Skocovska et al., 2007). No measurable adverse effects were observed in terms of mortality or clinical signs of disease following the 10-day and 30-day experiments (Skocovska et al., 2007). In a similar experiment, Damkova et al. (2009) exposed 40 2-month-old Japanese quail (20 breeding pairs) to MCs via dietary exposure, with MCs supplied in food provided to the birds daily for 8 weeks. They compared exposed quails to 20 breeding pairs (40 birds) that were not exposed to MCs as untreated controls. Mean daily consumption by exposed quails was 61.6 µg MCs (26.5 µg MC-RR, 7.6 µg MC-YR, and 27.4 µg MC-LR). No mortality or clinical symptoms of intoxication were observed in either control or exposed birds, and there were no differences in organ weights among unexposed and exposed birds (Damkova et al., 2009). Further, there were no differences in egg hatchability, eggshell thickness, body mass of chicks 14 days after hatching, or 14-day survival of chicks (Damkova et al., 2009). Since there were no apparent data regarding exposure-response relationships for birds and MC exposures, the SSD assembled for this study (Figure 3) does not contain data for avian species.

5.5 Toxicity data for plants and algae exposed to microcystins

Terrestrial plants can be exposed to MCs via irrigation water containing MCs, while aquatic plants and algae can be exposed directly in aquatic systems. Uptake of MCs into plant cells can occur via diffusion or root absorption, or direct contact of MC-laden water with leaves (i.e., via surface water or irrigation water) (Corbel et al., 2014). Pflugmacher et al. (2001) observed that uptake of MCs occurred through stems and rhizomes, and MCs were transported in xylem cells. Plants contain protein phosphatases 1 and 2A that play vital roles in molecular and physiological processes (Mackintosh et al., 1990; Takeda et al., 1994) that can be inhibited by MCs. Further, production of reactive oxygen species may result in oxidative stress within plant and algal cells (Pflugmacher, 2004; Corbel et al., 2014). In general, measured LOECs for plants and algae exposed to MCs range from 1 to 15 µg per L, in terms of decreases in growth (e.g., mass, shoot length, root length) relative to untreated controls (Figure 3; Table S6). Potency was well-demonstrated for lettuce, carrot, and green bean plants by Lee et al. (2017), as there were significant decreases in growth parameters (e.g., number of lettuce leaves per plant, diameter of carrot root, number of beans per plant) with relatively small changes in exposure concentration (1 to 10 µg MC per L). Outliers included responses of *Oryza sativa* and *Brassica napus*, for which LOECs were 2 to 3 orders of magnitude greater than for other plants tested (Chen et al., 2004). One possibility for this difference could be the manner in which seeds were exposed. Chen et al. (2004) exposed seeds by wetting paper with aqueous solutions of MCs and placing seeds on the wetted paper; in contrast, the majority of the other studies reviewed used seeds or plants that were often submerged or saturated with larger volumes of media containing MCs. These different methods of exposure could result in drastically different mass loading of MCs, uptake of MCs by plants, and ultimately manifested responses. Not only are plant exposures to MCs a concern for human health, but if adverse effects are elicited for crops, significant financial losses are possible (Corbel et al., 2014). Further, plants are a food source for animals, thus adverse effects to plants from MC exposures could result in negative consequences for higher organisms, either due to indirect exposures to MCs from ingestion of plants or lack of food available due to poor plant growth.

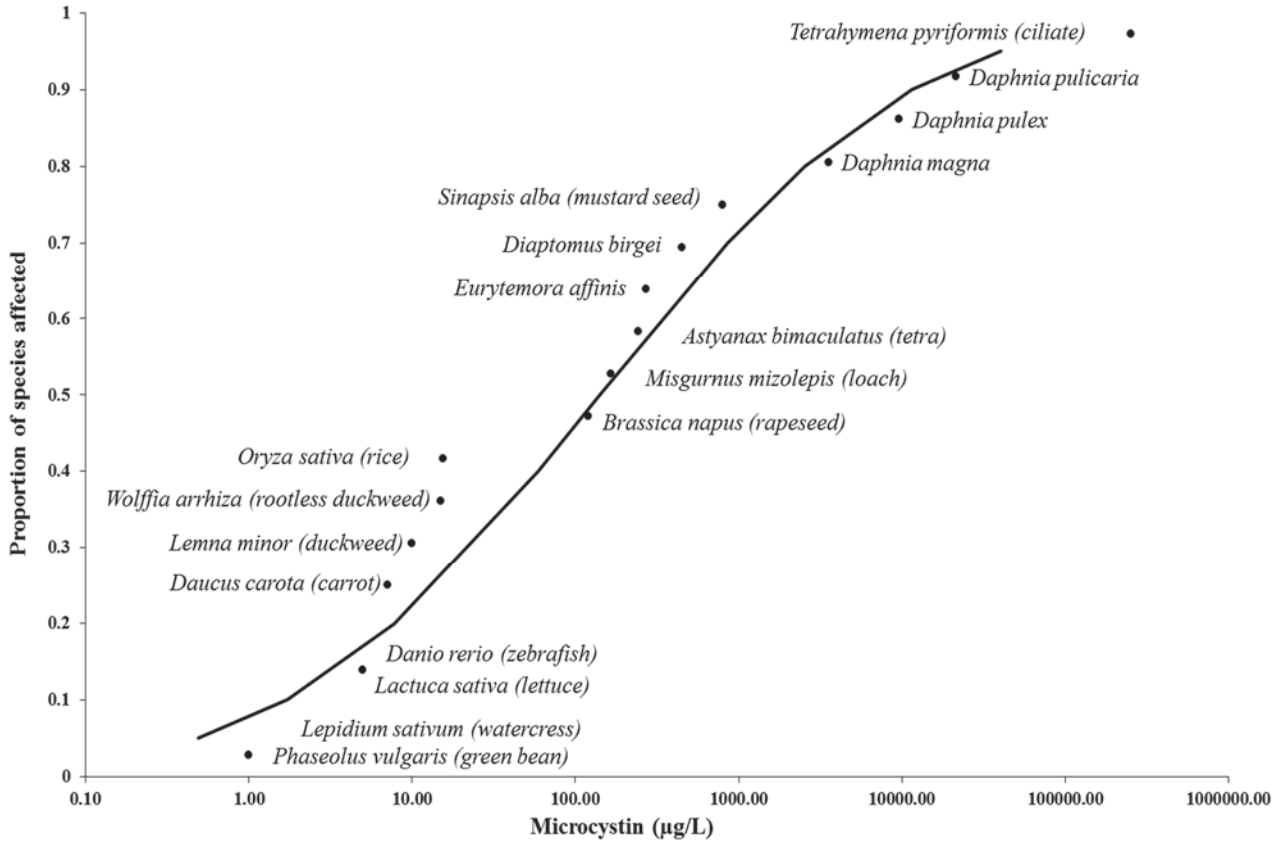

Figure 3. Species sensitivity distribution assembled from ecotoxicological data in peer-reviewed literature (data in supplemental material Tables S3-S6)

Based on ecological toxicity data assembled from peer-reviewed literature, in terms of relative sensitivities, plants ≥ fish > invertebrates. Data meeting specified criteria were not available for mammals or birds. However, there were data that qualified as secondary evidence that adverse effects from MC exposures have occurred for both mammals and birds. It should be noted that studies providing data used in the SSD for aquatic invertebrates mostly incorporated short-term exposure durations, which could result in over-estimated toxicity thresholds and false perceptions of insensitivity. Ideally, SSDs are assembled based on comparable experimental conditions to minimize confounding factors, yet since data are still limited for MC toxicity, the goal of this SSD was to provide a relative ranking of species sensitivity, while acknowledging data gaps. As more data are contributed to peer-reviewed literature, this SSD can be updated and improved.

Based on the assembled SSD, adverse effects are possible at MC exposures as low as 1 µg per L (Figure 3). The slope of the SSD curve (i.e., increase in number of species potentially affected with incremental increase in exposure concentration) shows that each order of magnitude increase from 1 µg MC per L (i.e., to 10 or 100) can impact a relatively large proportion (i.e., 30 to 40%) of the total distribution (Figure 3). To date, the majority of risk management efforts for MCs have focused on drinking water and recreation for humans. The ecological toxicity data reviewed in this study indicate that risk management is also needed for plants and animals. Ecological risk management is concerned with eliminating unnecessary adverse effects for populations, but in this context, adverse effects from MC exposures in pets, livestock, fish, birds, and endangered species can also directly and indirectly impact human health and economics.

6 Relative risks of no-action vs. action management decisions

The first few sections of this paper were intended to provide the foundational information needed to understand the risks associated with growth of microcystin-producing cyanobacteria in our freshwater resources. This information included the fundamental physical, chemical, and toxicological properties of microcystins, ways people can get exposed and the relative importance of each potential exposure route and source, and the relative sensitivities of various animals and plants to microcystins as reported in the peer-reviewed literature. The present section is focused on utilizing that information to compare the potential consequences of different management decisions from a high-level perspective.
In this paper, no action was defined as unabated growth of cyanobacteria and production of microcystins. This decision relies on the probability that a harmful algal bloom will dissipate naturally, which is an unpredictable outcome. Action decisions include exposure avoidance and control. Exposure avoidance was operationally defined as closure of water resources for drinking water or recreation in an effort to prevent human exposures. Exposure avoidance may be unenforced (e.g. where health advisories have been posted to suggest that individuals not enter water for recreation) or enforced (an enforced closure of a water resource for drinking water or recreation). Control was defined as techniques used in isolation or combination that result in timely and substantial decreases in microcystin-producing cyanobacteria and/or microcystins to levels that alleviate an existing or potential impairment to the uses or functions of the water resource. To compare each of these management decisions, possible outcomes in terms of potential risks and types of financial losses for each option were characterized.

Clearly, if no action is taken against microcystin-producing cyanobacteria, the potential for human, animal, and plant exposures exists. Based on the preceding information in this study, unmanaged exposures can lead to human, pet, and livestock illnesses and deaths, adverse impacts to aquatic ecosystems, lower crop yields, and risks to endangered and keystone species. No action can also result in a loss of designated uses of water resources (e.g. potable water, industry, irrigation, recreation, tourism, and aquaculture), which can result in severe financial losses. If exposure avoidance is employed, then human exposures may be avoided depending on the circumstance, but this decision does not account for ecological/agricultural risks nor the financial outcomes associated with the loss of the water's services. Finally, if a control method is employed (and is successful), avoidable exposures can be prevented, and designated uses of the water can be regained or maintained. Costs and collateral damage associated with a specific tactic should be evaluated in order to make an informed action decision.

Following characterization of exposures and potential effects of MCs, it is logical to compare the risks related to no-action and action decisions to discern whether the risks of MC exposures are sufficient to warrant management. To compare these decisions, the potential outcomes, in terms of risks and types of financial losses, of each option were characterized and compared. "No action" refers to unabated growth of cyanobacteria and production of MCs, so in terms of risk, no action can result in human, animal, and plant exposures and the potential for significant adverse effects as discussed previously. Financial losses are possible from surrendered uses of water resources, adverse effects to animals and plants, and losses in property values and tax revenues (Table 3). Dodds et al. (2008) estimated 2.2 billion dollars are lost annually as a result of cultural eutrophication in US freshwaters, with the greatest losses in property value and recreational use. Cultural eutrophication is implicated in several other water issues in addition to MC-producing cyanobacteria, but these estimates provide a metric of the types and magnitudes of financial losses that can be expected from cyanobacteria blooms. Types of financial losses associated with no action can also include illness or death for livestock and farm animals, lower crop yields, decline in tourism and recreation (swimming, boating, fishing), and the necessity of using bottled water in lieu of contaminated drinking water.

Exposure avoidance was defined as unabated growth of MC-producing cyanobacteria and MC production, coupled with a decision to close the water body for drinking water or recreational purposes (i.e., to prevent human exposures). Exposure avoidance may be unenforced, where health advisories have been posted in an effort to suggest that individuals not enter water for recreation (Ibelings et al., 2014). The decision to limit access to a water resource is typically based on a tiered framework with triggers based on cell density, MC concentration, or both (see Ibelings et al., 2014 for examples). This suggests that risk is relatively less when a warning is posted, as compared to a closure. However, as previously discussed, ***triggering management based on cell density can be flawed, since cell density frequently does not correlate well with MC concentrations***. When avoidance of a water resource infested with MC-producing cyanobacteria is indicated but not enforced, uses of the water resource may be partially maintained, but can also result in human exposures for individuals that do not heed warnings (Table 3). Enforced exposure avoidance (an enforced

closure of a water resource for drinking water or recreation) can result in reduced human exposures, but designated uses and services are then surrendered. Enforced exposure avoidance also assumes that all individuals avoid contact or ingestion of water, which may not be true. Neither type of exposure avoidance, as defined here, aims to prevent animal (wildlife or domestic) or plant exposures, meaning adverse effects are possible for those organisms. Further, many of the same types of financial losses can be expected as with no action outcomes, including illness and death of farm animals, lowered crop yields, lowered property values, and necessity of bottled water in lieu of contaminated drinking water, since no action is actually taken to alter exposures of MC-producing cyanobacteria or MCs.

Control is defined as techniques used in isolation or combination that result in timely and substantial decreases in MC-producing cyanobacteria and/or MCs to levels that alleviate an existing or potential impairment to the uses or functions of the water resource (adapted from Netherland and Schardt, 2012). In this context, techniques that decrease densities of cyanobacteria or MC concentrations are inherently decreasing potential for exposures and consequent adverse effects. Designated uses of water resources can be maintained or regained (e.g., drinking water, recreation, tourism, fish and wildlife propagation, irrigation, agriculture, and aquaculture) as a function of minimized exposures and adverse effects. Finally, financial losses previously described for no-action and prevention decisions can be significantly lowered, as property values can be restored, potential for risks to livestock and crops can be prevented, and recreation and tourism activities can be restored or maintained. Costs associated with control (for comparison to no-action costs) will be specific for the selected techniques discussed in the next section. Collateral damages resulting from the implemented control technique(s) should also be considered when making decisions. Clearly, there is potential for significant risks and financial losses associated with no-action against MC-producing cyanobacteria in freshwater resources, as well as current management approaches aiming to avoid exposures. Potential risks and financial losses could be alleviated if effective control techniques are implemented. Information is then necessary regarding risk management approaches available for MC-producing cyanobacteria (in-lake) and MCs in drinking water treatment facilities (in-plant).

Table 3. Potential outcomes associated with no-action, exposure avoidance, and control decisions

Risk management decision	**Potential outcomes**
No action	Unrestricted human exposures: gastrointestinal symptoms, skin rashes and irritation, eye and ear irritation, respiratory symptoms, liver failure
	Unrestricted livestock and pet exposures: gastrointestinal symptoms, weakness, liver failure, death, financial and personal losses
	Unrestricted ecological exposures: fish kills, bird mortalities, plant (crop) mortalities, risks to endangered and keystone species, financial losses
	Loss of designated uses of water: inability to provide services, financial losses
Exposure avoidance	***Unenforced***
	Potential for human and pet exposures: see effects above
	Unrestricted livestock and ecological exposures: see effects and financial losses above
	Enforced
	Unrestricted livestock and ecological exposures: see effects above
	Loss of designated uses of water: see financial losses above
Control	Avoidable human exposures prevented
	Avoidable domestic pet and livestock exposures prevented
	Avoidable ecological exposures prevented
	Designated uses of water resource maintained or regained
	Costs associated with control technique(s)
	Collateral damage associated with control technique(s)

7 Review of risk management approaches for microcystin-producing cyanobacteria and microcystins

The prior section of this paper provided high-level comparisons of potential outcomes associated with different management decisions (i.e., no action vs. action). In the scenario that a water resource manager concludes greater risk from the no-action management decision than taking action to intervene at their site, the next logical step is to select risk management approaches and develop a management plan. The goal of this section is to provide relevant data and other information that can help water resource managers identify risk management approaches for their site.

First, the site-specific problem can be defined, and management goals can be stated, so that a strategic plan for solving the problem and achieving management goals can be developed (discussed further in final section). Approaches for risk management of microcystin-producing cyanobacteria and microcystins can be selected based on management goals, site-specific characteristics, and available resources (personnel and budget). Approaches are categorized as "long term" and "short term", and according to whether the target is microcystin-producing cyanobacteria (in-lake) or microcystins (in-plant). In this context, long-term approaches are those applied to large scales (e.g. watershed), with expected results over the course of years, decades, or centuries. Short-term or "triage" approaches are applied locally (e.g. lake, reservoir, portion of lake or reservoir used for swimming, or drinking water treatment plant), for immediate mitigation of cyanobacteria and toxins, and restoration of designated water resource uses. In-lake approaches are intended to manage the cyanobacteria that produce microcystins, while in-plant approaches are intended to manage exposures of microcystins in cellular and/or aqueous forms. To support informed decision making, this section involves review of risk management approaches for microcystin-producing cyanobacteria and microcystins in terms of relative effectiveness, scalability, durability, and availability.

The well-known long-term approach of nutrient management in a watershed is first discussed in terms of available data on success stories. The focus of this review was on mitigation of phosphorus inputs to aquatic systems from external sources since this is the most widely used and promoted approach. A commonality found among successful case studies was that excessive inputs of phosphorus were from point sources (i.e., sources that could be identified and managed). Among these studies, response time in terms of decreased cyanobacteria biomass or chlorophyll-a concentrations ranged from 5-30 years and costs associated with the implemented management plans were up to billions of dollars. Some potential explanations for the relatively long response times for results could include internal loading of phosphorus (i.e., cycling from sediments), additional external loading of phosphorus (e.g. from non-point sources on land and atmospheric deposition from forest fires), uncontrollable external loading from extreme precipitation events resulting in pulse exposures of phosphorus in runoff, and human-induced changes in the watershed that could result in new inputs. The take-home message for this approach is that it should be carefully evaluated in terms of the probability of success, duration of time expected to achieve results, financial costs necessary to employ, and site-specific characteristics that could hinder success (e.g. sediment P concentrations, non-point sources).

Short-term risk management approaches were parsed as in-lake and in-plant, and further by physical, chemical, and biological. In-lake approaches are those targeted for management of MC-producing cyanobacteria. Physical in-lake processes reviewed in this section include aeration, coagulation/settling, and ultrasonication. Chemical in-lake processes include various algaecide formulations with active ingredients of copper, hydrogen peroxide, and endothall. Biological in-lake processes reviewed here include alteration of food web and introduction of barley straw. Regarding in-plant (drinking water treatment) approaches, physical methods include coagulation and flocculation, rapid sand filtration, sorption, and ultraviolet (UV) photolysis. Chemical approaches include oxidation with chlorine, permanganate, and peroxide, and advanced oxidation with ozone, TiO_2 photocatalysis, UV + peroxide, and ozone + peroxide. A biological approach reviewed is slow sand filtration, which involves biodegradation as the dominant treatment process. All of the above described approaches are reviewed in this section in terms of how they work and what the goal is, available performance data from the peer-reviewed literature, any limitations discussed in the literature, and any considerations that should be taken into account in terms of scalability, costs, selectivity, or otherwise.

To this point, exposures of MC-producing cyanobacteria and MCs, as well as potential exposure routes and effects for humans, animals, and plants, have been characterized. Following comparisons of potential risks and outcomes from no-action and action decisions, we conclude that the potential risks and outcomes associated with no-action are sufficient to warrant intervention. In a decision-making process for risk management, first, the site-specific problem should be

defined, and management goals should be stated, so that a strategic plan for solving the problem and achieving management goals can be developed (Huddleston et al., 2015).

For example, a hypothetical problem could be that MC-producing cyanobacteria have been identified in a drinking water reservoir in proximity to the drinking water intake structure, and total MC concentrations of 20 µg per L were measured. Since humans can be exposed to MCs from drinking water, there is potential for human health risks from colonization and growth of MC-producing cyanobacteria in the reservoir. The management goal can be stated as "total MC concentrations in finished (i.e., treated) drinking water are not to exceed the health advisory guideline or drinking water standard" (e.g. 0.3 µg per L; USEPA, 2015). Then, a strategic management plan can be developed to achieve this goal, which could involve one or more risk management approaches (i.e., tactics) for different targets (e.g., MC-producing cyanobacteria and MCs).

Approaches for risk management of MC-producing cyanobacteria and MCs can be selected based on management goals, site-specific characteristics, and available resources (Netherland and Schardt, 2012). Approaches are categorized as "long term" and "short term", and according to whether the target is MC-producing cyanobacteria (in-lake) or MCs (in-plant). In this context, long-term approaches are those applied to large scales (e.g., watershed), with expected results over the course of decades or centuries. Short-term or "triage" approaches are applied locally (e.g., lake, reservoir, portion of lake or reservoir used for swimming, or drinking water treatment plant) for immediate mitigation of exposures and restoration of water resource uses. In-lake approaches are intended to manage exposures of cyanobacteria that produce MCs, while in-plant approaches are intended to manage exposures of MCs, in cellular and/or aqueous forms. To support informed decision making, risk management approaches for MC-producing cyanobacteria and MCs were reviewed in terms of relative effectiveness, scalability, durability, and availability.

7.1 Long term risk management approach for MC-producing cyanobacteria

A proposed long-term risk management approach for MC-producing cyanobacteria involves decreasing or eliminating the mass loading of nutrients attributable to human activity (i.e., cultural eutrophication) to water resources. Total eutrophication is operationally defined as the sum of natural and cultural eutrophication. Natural eutrophication is the aging of aquatic systems, or nutrient enrichment of water over time (Schindler et al., 2016). Cultural eutrophication is human-accelerated aging of aquatic systems, which has been speculated to correlate with MC-producing cyanobacteria blooms, both spatially and temporally (Smith and Schindler, 2009; Carvalho et al., 2013).

The rationale often stated for decreasing cultural eutrophication in aquatic systems as a risk management approach is that cyanobacteria require nutrients for growth and survival. In several case studies, the frequency and severity of cyanobacteria blooms were decreased following alterations of mass loading per unit time of phosphorus that resulted in aqueous concentrations of 20-50 µg per L as total phosphorus (TP) (Phillips et al., 2005; Fastner et al., 2016; Schindler et al., 2016). However, overall, this long-term risk management approach for MC-producing cyanobacteria has been met with limited success. Internal loading of P (Wetzel, 2001; Phillips et al., 2005), external loading of P (e.g. non-point sources on land and atmospheric deposition from forest fires [Goldman et al., 1990]), extreme precipitation events resulting in pulse exposures of P from runoff, and human-induced changes in the watershed (e.g. land development) can result in unpredictable outcomes. Further, it is important to consider that some genera of cyanobacteria may be capable of growing in oligotrophic to mesotrophic systems, thus total P concentrations in the water column may not be the limiting factor in growth (Carey et al., 2008).

Inputs of TP come from point and non-point sources. Point sources can include municipal and industrial wastewater discharges, septic tank systems, and rural treatment plants, whereas non-point sources can include drainage from fertilized fields (e.g., farms, organic farms, and untreated sewage [e.g., combined sewage overflow, runoff from livestock waste]) in the watershed (Fastner et al., 2016). Sustained political will and stakeholder cooperation are necessary for success using this risk management approach. Efforts to decrease TP inputs to water resources from point sources are likely to achieve success more quickly than for water resources with mostly non-point sources (Fastner et al., 2016). Case studies that reported successful decreases in mass loadings of total P from point sources include Onondaga Lake and Washington Lake in the US, Lake Constance, Lake Tegel, and Schlachtensee in Germany, Lago Maggiore in Italy, and the western basin of Lake Balaton in Hungary (Fastner et al., 2016).

Techniques to decrease point-source loadings of total P have included upgrades in sewage treatment processes, removal of total P in tributary inflows to larger aquatic systems, and diversions of outflows from sewage treatment facilities to other aquatic systems often referred to as "less sensitive systems" (e.g., estuaries or oceans) (Fastner et al., 2016). A decrease in aqueous total P in Lake Constance (surrounded by Austria, Germany, and Switzerland) from 87 µg per L to 7.6 µg per L was achieved in approximately 28 years, following a capital investment equivalent to 4 billion US dollars from countries in the watershed targeted towards improvements in sewage and wastewater treatment facilities (Jochimsen et al., 2013). Decreases in cyanobacterial biomass from approximately 1 to 0.5 mg per L in the lake occurred after approximately 15 years (Jochimsen et al., 2013). In two lakes near Berlin (Schlachtensee and Lake Tegel), decreases of 2 to 3 orders of magnitude in aqueous total P concentrations were achieved within 5 years via P removal from tributaries (Schauser and Chorus, 2007). This treatment process cost approximately 0.18 euros (~ 22 US cents) per 1000 L treated in 1991 (capital + operating costs) and more recently 0.09 euros (~ 11 US cents) per 1000 L treated (Fastner et al., 2016). Phytoplankton biomass decreased significantly 4 years after treatment implementation in Schlachtensee, and 8 years after implementation in Lake Tegel (Schauser and Chorus, 2007). Edmonson and Lehman (1981) reported decreases in total P in Lake Washington from initial concentrations of 70 to 80 µg per L (between 1963 and 1967) to 20 to 30 µg per L (after 1968) after sewage effluent that would normally flow into Lake Washington was diverted into Puget Sound. While chlorophyll-a concentrations in the lake appeared to decrease on the same time scale as total P concentrations (e.g., 4 to 5 years), the proportion of cyanobacteria in total phytoplankton densities remained at 80 to 100% for 7 years following diversion of effluent, then decreased to approximately 20% (Edmonson and Lehman, 1981).

Overall, among successful case studies, the response time for substantial decreases in cyanobacteria densities following decreased phosphorus loadings from point sources has been approximately 5 to 30 years. Aggregate measurements of phytoplankton (e.g., biomass or chlorophyll-a concentration) often decline within several years of decreased total P loadings, but declines in the relative composition of cyanobacteria may require 2 to 3 times longer (Edmonson and Lehman, 1981; Phillips et al., 2005; Schauser and Chorus, 2007). Phillips et al. (2005) hypothesized that apparent lag times for cyanobacteria responses to decreased in-lake total P concentrations (e.g., 20 years in Barton Broad, UK) could be due to internal loading from sediments for years after aqueous inputs are altered.

Phosphorus has a lithic biogeochemical cycle, and exchange of P between sediments and water is a dominant process influencing P cycling in aquatic systems (Bostrom et al., 1988; Wetzel, 2001). Often, sediments contain P concentrations several orders of magnitude greater than the overlying water (Wetzel, 2001). Factors that influence P cycling among sediments and water include the retention ability for P in sediments, water characteristics, and biota inhabiting sediments that alter exchange rates (Wetzel, 2001). During summer stratification of aquatic systems, which is when the majority of cyanobacteria bloom events occur, anaerobic conditions at the sediment-water interface result in the release of P from sediments and bioavailability in water for phytoplankton (Bostrom et al., 1988; Paerl, 2014). The internal cycling of P has resulted in little or no success in many situations where external loadings of P were minimized, likely due to the retention and release of P in sediments (Bostrom et al., 1988). Further, there is potential for phytoplankton to directly utilize particulate P as a nutrient source once some critically low aqueous concentration is achieved. For example, particulate forms of P include organic P (bound to organic matter), inorganic P as the mineral apatite, and non-apatite inorganic P (absorbed to non-crystalline oxides, particularly iron) (Williams et al., 1976). Measurable by NaOH extraction, the non-apatite inorganic P strongly correlates with biologically available P, and is effectively taken up by algae (Santiago and Thomas, 1992). Santiago and Thomas (1992) reported that utilization by phytoplankton of particulate P (84% utilized was non-apatite inorganic P) occurred once soluble P decreased to less than 14 µg per L.

Data describing the successful decline in cyanobacteria biomass after P reduction all pertain to water resources for which the majority of total P inputs were from point sources. In these case studies, management goals were achieved on the order of years to decades with sustained financial efforts and stakeholder support. In order to achieve control over cyanobacteria colonization, decreases in total P loadings from both point and non-point sources are often needed (Fastner et al., 2016). In water resources that receive the majority of nutrient inputs from non-point sources (e.g., 71% for Lake Erie, US; Maccoux et al., 2016), sustained and persistent stakeholder agreement and cooperation will be necessary, which in the best-case scenario could lengthen the amount of time necessary to see results, or in the worst-case scenario could make management goals impossible to achieve. Focusing management goals on aqueous P concentrations does not account for internal cycling of P from sediments and does not account for the ability of phytoplankton to utilize particulate P as a nutrient source under limiting conditions in the aqueous phase. Further, focusing efforts on point sources of P does not account for rainfall events resulting in pulse exposures of P from runoff, or flushing of waters that

can “reset” total P concentrations in the aqueous phase. Thus, this approach should be carefully evaluated in terms of the duration of time expected to achieve results, the financial costs necessary, and site-specific characteristics (e.g., sediment P concentrations, non-point sources) that could hinder success.

Long-term nutrient control is an approach that aims to minimize MC-producing cyanobacteria in critical freshwater resources over time. However, even if successful, nutrient control is not a method capable of managing exposures or restoring designated uses of critical water resources in the short term. When short-term and long-term approaches are combined in an integrative management plan, short-term approaches can alleviate potential risks and financial losses in high-pressure situations that require immediate action, while long-term nutrient management may decrease the potential for these issues in the future.

7.2 Short term risk management approaches for MC-producing cyanobacteria and MCs

Short-term risk management approaches for MC-producing cyanobacteria are based on altering physical conditions in the water resource (i.e., in-lake) such that cyanobacteria cannot colonize, physically settling planktonic cells to sediments (indirectly eliciting adverse effects), or directly eliciting adverse effects to cyanobacteria sufficient to stop MC production. Risk management approaches for MCs specifically are implemented in drinking water treatment facilities (i.e., in-plant) and targeted for removal of cellular and aqueous MCs to prevent human exposures from drinking waters. Approaches for MC-producing cyanobacteria and MCs are parsed as physical, chemical, and biological (Table 4).

Table 4. Short-term risk management approaches for MC-producing cyanobacteria and MCs

	Target		
Type	**MC-producing cyanobacteria (in-lake)**	**MCs (in-plant)**	
Physical	Aeration	Coagulation/flocculation	
	Coagulation and settling	Rapid sand filtration	
	Ultrasonication	Sorption (GAC & PAC)	
		UV photolysis	
Chemical	Algaecides:	Oxidants:	Advanced oxidation:
	Copper-based formulations	Chlorine	Ozone
	Hydrogen peroxide-based formulations	Potassium permanganate	TiO_2 photocatalysis
	Endothall formulation	H_2O_2	UV + H_2O_2
			Ozone + H_2O_2
Biological	Alteration of food web structure	Slow sand filtration (biodegradation)	
	Barley straw		

7.3 Short term risk management approaches targeted for MC-producing cyanobacteria (in-lake)

7.3.1 Aeration as a physical risk management approach for MC-producing cyanobacteria

The often-stated goal of aeration devices is to mix water and disrupt stratified conditions in a water resource, such that planktonic cyanobacteria that form surficial scums cannot remain at the water surface. Thermal destratification from aeration-induced mixing has resulted in substantial decreases in cell densities of cyanobacteria, often in relatively small water bodies with deep (>5 m) water columns (Burns, 1994; Jungo et al., 2001; Heo and Kim, 2004). Aeration will likely not be successful for mitigating MC-producing cyanobacteria in shallow (< 5 m) water, since light can be sufficient at all depths for cyanobacterial growth (Lackey, 1973; Jungo et al., 2001). In addition, aeration may not be possible in large reservoirs given the energy required to mix the entire volume of water (Paerl, 2014). Aeration would not be effective for benthic cyanobacteria (e.g., *Oscillatoria*), since mixing would likely create a continuous flow of aqueous nutrients for those species. Aeration does not alter the trophic state (e.g., total nitrogen, total phosphorus, or chlorophyll-a concentrations; Cowell et al., 1987; Heo and Kim, 2004), which led to hypotheses that light limitation is the cause of cyanobacteria decline from destratification (Jungo et al., 2001). Alternatively, Burns (1994) hypothesized that year-round oxygenation prevented reducing conditions in sediments that could liberate phosphorus into the water and promote growth of cyanobacteria.

Aeration can result in a shift in composition from a majority of cyanobacteria to chlorophytes (i.e., green algae) and diatoms, since mixing of waters minimizes losses of these species to sedimentation that would normally occur in unmixed waters (Jungo et al., 2001; Heo and Kim, 2004). Given that several genera of diatoms are capable of producing taste and odor compounds (e.g., 2-methylisoborneol and geosmin) that can be aesthetically displeasing for humans, careful monitoring of species composition following use of aeration would be useful. The pressure and volume of air required per unit time to destratify a water body depends on the degree of stratification prior to mixing, volume of water, and surface area of the water resource (Knoppert et al., 1970). Multiple devices can be installed throughout a system to achieve the total amount of mixing necessary. All areas of a water body must be well-mixed or cyanobacteria can float to more stagnant areas (Visser et al., 1996). Measurements of dye dispersal in water, as well as water temperature and dissolved oxygen profiles, can be conducted to confirm homogeneous mixing throughout a system. Aeration must be specifically designed for each site to ensure adequate energy is supplied to the entire system, and to avoid lack of performance due to insufficient mixing (Osgood and Stiegler, 1990). Total costs for aeration would include capital costs for equipment and installation, and yearly costs for maintenance and energy expended (Burns, 1994). More recently, solar powered aeration devices have been developed and employed in full-scale operations, which can limit expenses incurred from energy requirements (Hudnell et al., 2010).

7.3.2 Coagulation and settling as a physical management approach for MC-producing cyanobacteria

Coagulation is a process by which the negative charges on algal cells are neutralized such that particles (i.e., cells) that normally repel each other and remain suspended in the water column instead aggregate and form larger particles that can settle to the sediments (Huh and Ahn, 2017). Coagulants are not directly algaecidal, rather they are often used to increase clarity in water. Therefore, the process of coagulation and settling does little to mitigate MC-producing cyanobacteria from a water resource, although indirect algaecidal effects to planktonic cyanobacteria may occur due to light limitation. If coagulants are used for their algaecidal properties and are not registered as algaecides, the legality of this approach is often questioned. Inorganic coagulants that have been used to settle cyanobacteria and algal cells to sediments include clays (e.g., kaolinite, illite, and Ca-montmorillonite), aluminum sulfate, ferric chloride, and lime (Huh and Ahn, 2017). For any coagulant, effectiveness depends on the proportion of binding sites relative to the number of cyanobacteria cells that require precipitation (Huh and Ahn, 2017). Therefore, the mass of coagulant necessary is site specific. Clay coagulants have been used for effective decreases in visible blooms of marine dinoflagellates and haptophytes in Japan, South Korea, China, the United States, Sweden, and Australia (Sengco and Anderson, 2004). No studies were found for precipitation of cyanobacteria in freshwater resources using coagulation. Further, smaller-sized cells with relatively higher surface areas (e.g., cyanobacteria) may require proportionally higher quantities of coagulants for settling (Guenther and Bozelli, 2004). Clay-enhanced settling of cyanobacteria cells can also result in pulse loadings of organic matter to the benthic regions of water resources, potentially resulting in anaerobic conditions at the sediment-water interface and subsequent release of P (Paerl et al., 2016).

Aluminum and ferric salts have also been used as in situ coagulants with limited durability. For example, Lelkova et al. (2008) observed an immediate decline in cell density of *Planktothrix agardhii* after the addition of 5.3 mg alum per L to the infested water resource, but several weeks later, cells began to proliferate. Adverse effects on zooplankton have been observed following alum applications, which may be due to adhesion of agglomerated particles to their filter apparatus, decreased availability of food (e.g., phytoplankton removal from the water column), entrapment in flocculated particles, or potential bioavailability of aluminum as pH decreases during hydrolysis of aluminum (Jancula and Marsalek, 2011; Jancula et al., 2011). Ferric salts also act as coagulants for algal cells (Jiang et al., 1993; Chow et al., 1998), but have rarely been used in aquatic systems due to redox activity and the potential for decreased pH in water due to rapid hydrolysis. Overall, coagulants do not decrease densities of MC-producing cyanobacteria in aquatic systems; rather, they settle cells from the water column to the benthic areas. Since coagulants transfer cyanobacteria cells, rather than remove cells from aquatic systems, the durability of this approach is likely minimal, but could be temporarily effective if immediate decreases in total suspended solids and/or turbidity is the management goal. If settled areas in the water resource are not light limited, there is potential for continued growth of cyanobacteria and production of MCs; if light is limited, there is potential for rapid senescence and lysis of cells.

7.3.3 Ultrasonic energy as a physical management approach for MC-producing cyanobacteria

Cyanobacteria contain gas vacuoles that regulate cell buoyancy, which is a competitive physiological characteristic (Graham and Wilcox, 2000). The goal of ultrasonic energy is to minimize competitive characteristics in cyanobacteria that support rapid growth and colonization. Ultrasound waves can contain frequencies higher than 20 kHz, resulting in structural disruption of cyanobacteria cells (Phull et al., 1997). Following rupture of gas vacuoles in cells, adverse effects on cell membranes, photosynthetic activity, and cell division have been observed (Rajasekhar et al., 2012). The effectiveness (i.e., extent of control) of ultrasonication depends on the frequency of waves introduced into the water (in kHz), the ultrasonic dose (e.g., the quantity of energy supplied per unit volume of water), and the duration of exposure (Rajasekhar et al., 2012). There have been conflicting interpretations of effective frequencies; for example, some suggest lower frequency (i.e., 10 kHz) to minimize the amount of energy required for production of cavitation bubbles (Rajasekhar et al., 2012), while Joyce et al. (2010) observed greater decreases in cell densities at higher frequencies (e.g., 864 kHz). At higher frequencies, Joyce et al. (2010) attributed adverse effects in *Microcystis aeruginosa* to the mechanical effects of cavitation bubbles, as well as the production of free radicals following decomposition of water induced by cavitational collapse.

The majority of peer-reviewed publications evaluating ultrasonication to control cyanobacteria are from studies conducted in laboratory settings. In one field study, ultrasonic energy implemented in a relatively small drinking water reservoir in Short Hills, NJ (USA) was effective for decreasing initial cell densities of approximately 10^4 cells per mL of *Aphanizomenon* to non-detectable levels within approximately 8 days; however, when treatment ceased, cell densities increased almost immediately (Schneider et al., 2015). Four devices were distributed across the reservoir, with each having a targeted range of up to 500 m according to Schneider et al. (2015). Costs associated with this approach would include capital costs (or leasing costs) for equipment and installation, and yearly costs from energy expended and maintenance, which would be specific to the size of the site. For reservoirs with relatively large surface areas, ultrasonication may be impractical given the amount of energy that would be necessary to cover the entire surface area. Laboratory studies have shown that cells can regenerate gas vacuoles within 24 hours of sonication treatment, when supplied with adequate light and aeration (Lee et al., 2000), further supporting the notion that continuous ultrasonication is necessary for control during the growing season.

7.3.4 Algaecides as chemical risk management approach for MC-producing cyanobacteria

Algaecides are used to control and suppress colonization of cyanobacteria in surface waters, and can be particularly useful in situations where immediate mitigation of cyanobacteria is necessary. Algaecides for control of cyanobacteria contain active ingredients of copper, hydrogen peroxide, and endothall. Diquat, carfentrazone-ethyl, and flumioxazin are active ingredients in registered herbicides that are, in some cases, labeled for control of filamentous eukaryotic algae, but are not currently instructed for control of cyanobacteria.

Formulations of copper-based algaecides include copper sulfate pentahydrate and several chelated copper forms including copper citrate and copper gluconate (e.g. Algimycin® PWF; Applied Biochemists, 2020a), copper ethanolamine and triethanolamine (e.g. Cutrine® Plus and Captain®; e.g. Applied Biochemists, 2020b; SePRO, 2020a), and copper ethanolamine and triethanolamine with adjuvents to help penetrate mucilage coatings on cells (e.g. Cutrine® Ultra and Captain® XTR, Applied Biochemists, 2020c; SePRO, 2020b). Formulations of hydrogen peroxide-based algaecides include granular sodium carbonate peroxyhydrate (SCP)(e.g. Phycomycin SCP and GreenClean® Pro; Applied Biochemists, 2020d; BioSafe Systems, 2020a) and hydrogen peroxide with peroxyacetic acid (e.g. GreenClean® Liquid 5.0; BioSafe Systems, 2020b). Endothall is registered as an algaecide in the form of an alkylamine salt (e.g. Hydrothol® 191; United Phosphorus Limited, 2020). Several algaecides are certified by the National Sanitation Foundation for use in drinking waters. Algaecides can also be used in irrigation waters and recreational waters, and can be applied by licensed and certified algaecide applicators with required permits (requirements vary by state). The necessary frequency and intensity of algaecide exposures are site-specific, and repeated applications are often necessary (within a growing season and from year to year) to maintain control of cyanobacteria. Availability of this approach can depend on obtaining a permit for application (which can take days to weeks), availability of licensed applicators for application at the appropriate time, and availability of algaecide for delivery to the site within the needed time frame.

Copper has been used as an algaecide for over a century (Moore and Kellerman, 1905) and is well-characterized in terms of relative effectiveness for mitigating cyanobacteria, margins of safety for non-target species, and environmental fate. Chelated copper algaecides are formulated to minimize complexation with dissolved anions in water and increase bioavailability to algal cells. Cyanobacteria are relatively sensitive to copper-based algaecides compared to eukaryotic algae, fish, and benthic invertebrates (Murray-Gulde et al., 2002; Calomeni et al., 2014; Geer et al., 2016). Water characteristics including hardness, pH, alkalinity, and conductivity can influence bioavailability of copper to target and non-target species (de Schamphelaere and Janssen, 2002; Rodgers et al., 2010). For example, as hardness, pH, alkalinity, and conductivity of water decline, toxicity thresholds also decrease. Half-lives for copper in the aqueous phase following pulse exposures from algaecides can range from minutes to days (Button et al., 1977; Murray-Gulde et al., 2002; Anderson et al., 2003; Calomeni et al., 2017), and depend on site-specific fate processes (e.g., dilution, dispersion, sorption to algal cells, sorption to sediments), where the dominant fate process at a site will likely influence exposure duration (Calomeni et al., 2017). Since copper has a lithic biogeochemical cycle, it is commonly claimed in the peer-reviewed literature that copper accumulates in sediments to concentrations that elicit toxicity in benthic invertebrates. However, the studies often cited for this claim have been conducted in small impoundments (e.g., farm dugouts and catfish ponds) that were representative of "whole pond treatments" (Prepas and Murphy, 1988; Liu et al., 2006). A maximum of half of the total surface area of a water resource can be treated at one time, and treatments cannot be conducted more often than every 14 days according to copper algaecide labels in the US (e.g. Applied Biochemists, 2020b; SePRO 2020a). Further, as previously mentioned, sediment sorption is not the sole fate process for copper following pulse exposures of copper-based algaecides. Following recent (i.e., several days past; Calomeni et al., 2015) and repeated (i.e., over the course of 7 to 20 years; Iwinski et al., 2016b) copper algaecide applications, there were no differences in sediment copper concentrations between untreated and treated coves in a southeastern reservoir, nor were adverse responses measured in laboratory-cultured benthic invertebrates exposed to treated sediments. Further, there were no differences in diversity or relative abundance of benthic invertebrates collected from sediments in untreated and treated coves (Iwinski et al., 2016b).

The concentration of copper necessary to achieve control of MC-producing cyanobacteria decreases with decreasing cell density (Kinley et al., 2017). Further, concentrations of copper algaecides that can control cyanobacteria are lower than concentrations that result in maximum MC release from cells (Iwinski et al., 2016c; Kinley et al., 2017). Therefore, with knowledge of exposure-response relationships and rate of growth of cells for site-specific cyanobacteria, copper-based algaecides can be used strategically to mitigate cell densities while minimizing the extent of MC release, if that is of concern (e.g., in reservoirs used for drinking water). When cyanobacteria are exposed to copper algaecides in a targeted manner, exposures can be minimized for non-target organisms. For example, surficial injections of algaecides can be used to expose buoyant planktonic cyanobacteria to algaecides, while drop hoses can be used to expose cyanobacteria in benthic areas.

Hydrogen peroxide-based algaecides are relatively new (i.e., < 20 years) with formulations as sodium carbonate peroxyhydrate (SCP) and hydrogen peroxide with peroxyacetic acid. Peroxide-based algaecides dissociate rapidly into oxygen and water following introduction in aquatic systems (e.g. half-lives < 1-d; Geer et al., 2017). Data are lacking in the peer-reviewed literature for full-scale performance of hydrogen-peroxide based algaecides in controlling MC-producing cyanobacteria blooms. Based on what is known from laboratory studies, cyanobacteria are relatively sensitive to these algaecides and there are large (i.e., 1-2 orders of magnitude) margins of safety for eukaryotic algae and fish (Schrader et al., 1998; Drabkova et al., 2007; Geer et al., 2016). For example, the 96-h EC50 for control of *Microcystis aeruginosa* in terms of cell density (0.9 mg H_2O_2/L as a granular SCP algaecide) was an order of magnitude less than the EC50 for a eukaryotic alga, *Pseudokirchneriella subcapitata*, and two orders of magnitude less than the LC50 for < 24-h old fathead minnows (*Pimephales promelas*) (Geer et al., 2016). As for other algaecides, it is necessary to achieve sufficient exposure concentration and contact duration with cyanobacteria to achieve adverse effects.

Endothall is registered as an algaecide as a dimethylalkylamine salt produced in liquid and granular forms. As for copper and hydrogen peroxide-based algaecides, cyanobacteria are often more sensitive than eukaryotic algae to exposures of the dimethylalkylamine salt of endothall (Ruzycki et al., 1998). For 96-hour laboratory exposures, EC_{50} values in terms of cell density of two strains of *Microcystis aeruginosa* were approximately 0.07 and 0.11 mg endothall acid equivalent (a.e.) per L, compared to 96-hour EC_{50} values of 0.27 and 0.42 mg a.e. per L for the chlorophytes *Chlamydomonas noctigama* and *Scenedesmus acuminatus*, respectively (Ruzycki et al., 1988). In laboratory toxicity experiments with 7-day exposure durations, Spencer et al. (2013) observed decreased growth of the cyanobacterium *Nostoc* at an exposure

concentration of 0.3 mg endothall a.e. per L. However, in a field-scale experiment, no measurable adverse responses in *Nostoc* were observed up to 5 mg endothall a.e. per L, which was attributed to the addition of microbial assemblages from rice straw in the field that were capable of rapidly degrading endothall, thus resulting in a shortened exposure duration as compared to laboratory experiments (Spencer et al., 2013). Biodegradation is the dominant fate process influencing exposure durations of endothall in aquatic systems (Westerdahl and Getsinger, 1988), so environmental conditions influencing growth of heterotrophic bacteria (e.g., nutrients, water temperature, dissolved oxygen) likely influence exposure durations of endothall indirectly. For endothall-based algaecides, LC_{50} values for several freshwater fish (e.g., rainbow trout, bluegill sunfish, and golden shiner) and the aquatic invertebrate *Daphnia magna* range from approximately 0.3 to 1.3 mg endothall a.e. per L (Elf Atochem, 1990). Therefore, a cautious approach is needed to ensure that the exposure concentrations needed to control cyanobacteria do not harm off-target organisms; algaecides should be applied to the algae and not to the water column, using broadcast sprays or surficial injections for surficial scums or drop hoses for benthic cyanobacteria.

Certain food-grade dyes (e.g., Aquashade™) are registered as algaecides by the USEPA, and are intended to shade light from photosynthetic organisms. When used according to the label, the algaecide can result in a pale blue color in water resources, and is typically used at concentrations of 1 mg per L or less for control of algae (Madsen et al., 1999). The label of Aquashade™ states that the product should not be used in waters used for human consumption or in water resources that are not under total control of the user (e.g., streams, rivers, and reservoirs). Use of this approach may be appropriate in small ponds and recreational areas that have little or no outflow of water.

7.3.5 Alteration of food web structure as a biological risk management approach for MC-producing cyanobacteria

In the scientific literature, several approaches are described that aim to alter the food webs of aquatic systems, to increase grazing pressure on cyanobacteria, or decrease cycling of nutrients. In one approach, the abundance of herbivorous zooplankton that can feed on cyanobacteria is increased, either by removing planktivorous fish or adding piscivorous fish (Shapiro et al., 1983). In a second approach, benthivorous fish are removed to decrease cycling of nutrients from the sediments (Shapiro et al., 1983; Paerl, 2014). Following targeted mortality of planktivorous fish using exposures of rotenone, populations of zooplankton (e.g., *Daphnia pulex*) increased, and subsequently algal biomass decreased in several aquatic systems (Shapiro et al., 1983). However, the effects are less significant in hypereutrophic shallow systems (Xie and Liu, 2001), and the durability of this approach is questionable, given that dominance of MC-producing cyanobacteria could result if zooplankton selectively feed on non-toxic strains (Paerl, 2014). Many cyanobacteria may also have characteristics that prevent grazing by zooplankton, including mucilaginous sheaths (e.g., colonial *Microcystis*) (Xie and Liu, 2001). Toxicity to aquatic invertebrates from exposures of MCs, as well as bioaccumulation of MCs in aquatic biota, are potential consequences of this approach, if it is somewhat successful. Although increased pressure of zooplankton grazing may be effective for non-toxin-producing cyanobacteria and other problematic algal species, it is likely not logical or effective in this context, given that it provides an avenue for bioaccumulation in aquatic food webs, and overall, likely does not result in rapid or long-term control of MC-producing cyanobacteria. Xie and Liu (2001) concluded that stocking of carp (e.g., silver carp and bighead carp) resulted in rapid and continued mitigation of cyanobacteria blooms, given that fish biomass remained around 50 g per m^3. However, based on what is known regarding MC toxicity to fish (Table S4), it is likely not logical to use planktivorous fish as herbivores for MC-producing cyanobacteria.

7.3.6 Barley straw as a biological risk management approach for MC-producing cyanobacteria

Barley straw (*Hordeum vulgare*) has apparent algaestatic properties for cyanobacteria (e.g., *Microcystis aeruginosa*) which may be due to polyphenolics with molecular weights ranging from 1000 to 3000 Da (Waybright et al., 2009). The effect of rotting barley straw on growth rate of cyanobacteria and algae has had mixed results in laboratory and field studies. For example, Cheng et al. (1995) observed no algaestatic or algaecidal effects against *Microcystis aeruginosa* over a 6-month period in a field-scale experiment using 6 experimental ponds. In a laboratory experiment, Jelbart (1993) did not identify adverse effects from rotting straw on an isolated strain of *Microcystis aeruginosa*. Alternatively, Everall and Lees (1996) described significant decreases in both cyanobacterial dominance and phytoplankton productivity in reservoir trials using 50 g of barley straw per m^3. To date, the specific compounds responsible for algaestatic effects and the modes of action are poorly studied and not well-understood. Thus, the reliability of outcomes is questionable. In addition, if there appears to be algaestatic or algaecidal properties resulting from exposures to a certain compound, it

could be a source of risk to other organisms. Introduction of organic matter into aquatic systems could result in anaerobic conditions (e.g., from 50 g of barley straw per m^3 as used by Everall and Lees, 1996) that could be detrimental to non-target organisms or result in release of P from sediments, especially in shallow or stagnant waters.

7.4 Short term risk management approaches for microcystins in drinking water treatment (in-plant)

7.4.1 Coagulation, flocculation, and sedimentation as physical risk management approaches for microcystins

As previously described, coagulation is the process by which charges on molecules are neutralized, minimizing forces that repel particles from other particles, and allowing particles to agglomerate. Flocculation involves gentle mixing to increase rates of agglomeration, to form larger particles that can be easily removed via sedimentation or filtration (Svrcek and Smith, 2004). In drinking water treatment, aluminum and ferric iron salts, as well as synthetic polymers, are used to coagulate algal and cyanobacterial cells. For example, Chow et al. (1999) measured > 99% removal of *Microcystis* cells (from initial cell density of 10^5 cells per mL) using an exposure of 5.8 mg per L of aluminum sulfate for 6 hours in pilot scale experiments, and there was no measurable lysis of cell membranes from this treatment. When ferric chloride was used as a coagulant, Chow et al. (1998) measured increases in cell densities of *Microcystis* and *Anabaena*, and it was hypothesized that if iron is limiting in source waters, use of an iron-based coagulant may stimulate growth of cyanobacteria. However, in several other experiments, ferric chloride has been as effective as alum-based coagulants for removal of cyanobacteria (Drikas et al., 2001), and laboratory-scale experiments can be useful for determining effective coagulant, concentration, and contact duration for each specific plant.

Following coagulation and flocculation, waters are clarified prior to filtration. This can be achieved via sedimentation or dissolved air flotation (Drikas et al., 2001; Svrcek and Smith, 2004). Sedimentation is the process by which agglomerated particles settle to the bottom of the clarifying vessel, accumulating sludge that routinely requires removal from the system. In waters with relatively high densities of algae or cyanobacteria, particulates require longer to settle (Mouchet and Bonnelye, 1998), and therefore dissolved air flotation may be more effective for cyanobacteria cells, especially due to their tendency to float to the water surface from gas vacuoles (Letterman, 1999; Drikas et al., 2001). Further, settled cyanobacteria cells have potential to lyse (Drikas et al., 2001), likely due to apoptosis influenced by light limitation. Therefore, dissolved air flotation may be a more effective approach for physical removal of intact cyanobacteria cells following coagulation and flocculation (Edzwald, 1993; Vlaski et al., 1996; Hrudey et al., 1999).

7.4.2 Rapid sand filtration as a physical risk management approach for microcystins

Given that some level of physical removal of cells occurs prior to filtration (e.g., coagulation, flocculation, and clarification), rapid sand filtration can be used to remove remaining cyanobacteria cells from solution. Filtration alone is not recommended for physical removal of cellular MCs, since filter pore sizes that are sufficiently large to prevent clogging will likely not retain cyanobacteria cells, and conversely, pore sizes that are small enough to capture cyanobacteria are susceptible to rapid clogging that would require frequent backwashing (Drikas et al., 2001). To maintain efficiency in treatment, filtration is more likely to be useful as a “polishing step” rather than the only barrier for cellular MCs. Hoeger et al. (2004) observed 98 to 99% removal of cyanobacteria cells via flocculation; however, percentages can be misleading in the context of cell densities of algae or cyanobacteria, as a 99% decrease from a cell density of $1.5x10^5$ cells per mL results in a cell density of $1.5x10^3$ cells per mL. Hoeger et al. (2004) noted that remaining cell densities of greater than 3000 cells per mL were often measured following flocculation, clearly necessitating frequent measurements of total MCs between treatment steps, and additional processes to remove residual cell densities prior to processes targeted for treating aqueous MCs.

7.4.3 Powdered and granulated activated carbon as physical risk management approaches for microcystins

In drinking water treatment plants, powdered activated carbon (PAC) and granulated activated carbon (GAC) physically remove organic constituents via adsorption. PAC is added to raw water during filtration or coagulation, while GAC is often used in flow-through columns (Lawton and Robertson, 1999). Wood and coal-based carbons are more effective for removal of MCs than coconut-based PAC or GAC (Donati et al., 1994; Mohamed et al., 1999; Cook and Newcombe et al., 2002; Campinas and Rosa, 2006). Wood-based activated carbon is mesoporous (i.e., pore size 2 to 50 nm) with relatively large pore volumes, which is ideal for large molecules like MCs (800 to 1000 Da) that will not effectively sorb

to microporous carbons (Donati et al., 1994). For PACs, the concentration amended to water has a stronger influence on rate and extent of MC removal than contact time beyond a threshold contact duration (e.g., 30 minutes). Concentrations of PAC ranging from 15 to 100 mg per L have been effective for decreasing MC concentrations to less than 1 µg per L, depending on the relative composition of MC congeners (Donati et al., 1994; Cook and Newcombe et al., 2002; Ho et al., 2011).

In general, in order of rate and extent of removal via sorption to wood and coal-based PACs, MC-RR > MC-YR > MC-LR > MC-LA (Cook and Newcombe, 2002; Ho et al. 2011), which is counterintuitive to predicted outcomes, given that MC-RR is hydrophilic and MC-LA is hydrophobic. These data suggest the surface charge of MCs at environmentally relevant pH plays a role in sorption effectiveness to activated carbons. For example, at pH 6 to 8.5, MC-RR carries a net neutral charge, whereas MC-LR carries a net -1 charge, and MC-LY, MC-LW, and MC-LA each carry a net -2 charge (Cook and Newcombe, 2002; Campinas and Rosa, 2006), which may explain why MC-RR is frequently removed rapidly and to the greatest extent among congeners of MCs under the same exposure conditions (Cook and Newcombe, 2002; Ho et al., 2011). Therefore, relative sizes of MC molecules and adsorbant pores are dominant factors influencing the rate and extent of sorption of total MCs overall, but specific surface charges can influence effectiveness among congeners. Dissolved organic carbon can also influence performance, since organic molecules can compete with MCs for sorption sites on activated carbon (Donati et al., 1994; Cook and Newcombe, 2008).

Mohamed et al. (1999) found that PAC was more effective than GAC at removing total MCs for a contact duration of 7 days at activated carbon concentrations ranging from 100 to 500 mg per L; however, PAC concentrations and contact durations actually used in drinking water treatment plants are much lower than those evaluated in their study. Therefore, based on feasible design conditions in most situations, GAC may be more effective, and has been in several studies (Keijola et al., 1988; Falconer et al., 1989; Himberg et al., 1989). The installation of GAC filtration beds is common in modern treatment facilities, yet the lifetime of the GAC (duration of effective MC adsorption without breakthrough) is relatively short (e.g., less than 5 months) (Lawton and Robertson, 1999), and costs associated with re-charge are substantial.

Installations of PAC and GAC for removal of aqueous MCs can be effective given that design is tailored to the specific site characteristics. An advantage of this approach is that it is already widely used in drinking water treatment plants, thus availability and scalability are clearly adequate. Treatment design parameters including type and quantity of activated carbon and contact duration are site specific (influenced by site water pH and DOC concentration, concentration of aqueous MCs requiring removal following physical removal of cellular MCs, and qualitative composition of MCs), and can be experimentally determined using laboratory-scale experiments prior to installation (see AWWA, 2018 for testing protocols). Since site characteristics likely to influence performance can shift with time, it is logical to design a robust system to ensure treatment under “worst-case conditions” (e.g., composition of MCs mostly those congeners with a net negative charge, relatively high DOC concentrations).

7.4.4 UV photolysis as a physical risk management approach for microcystins

Since MCs absorb light in the UV range, it is logical that UV photolysis could be effective for transformation of MCs to non-toxic compounds (Sharma et al., 2012). Tsuji et al. (1995) measured a half-life of approximately 10 minutes for MC-LR exposed to 147 µW per cm^2 lamp emitting light at 254 nm. When irradiance was increased to 2550 µW per cm^2, 100% of MC-LR was removed in 10 minutes (Tsuji et al., 1995). Qiao et al. (2005) measured a half-life of approximately 15 minutes using a UV lamp emitting 254 nm wavelength light at 3.66 mW per cm^2 and an initial MC-RR concentration of 720 µg per L. There were no pilot or full-scale experiments found for UV photolysis of MCs. Based on laboratory experiments, the necessary UV irradiance for photolysis of MCs would be several orders of magnitude greater than irradiation typically used for decontamination in drinking water treatment plants (Svreck and Smith, 2004; de la Cruz et al., 2011). Therefore, UV alone may not be an efficient approach (due to electricity costs), but could be useful when combined with other approaches, as will be discussed further in a subsequent section of this paper regarding combined advanced oxidation approaches.

7.4.5 Oxidation with chlorine as a chemical risk management approach for microcystins

Disinfection of drinking waters using chlorine has been commonplace since the beginning of the 20th century (Lawton and Peterson, 1999). In the context of MC removal, pre-oxidation processes (i.e., before filtration and coagulation processes) should be avoided to minimize cell lysis and release of aqueous MCs into water (Tsuji et al., 1997; Lawton and Peterson, 1999; Svrcek and Smith, 2004; Daly et al., 2007). Rates of oxidation of MCs from exposures to chlorine depend on pH and chlorine concentration (Acero et al., 2005; Daly et al., 2007). For example, Acero et al. (2005) measured 2nd order rate coefficients for total MCs (sum of congeners) of 475 M (mol per L)/per second at pH 4.8 and 9.8 M per second at pH 8.8. For oxidation of MC-LR specifically, half-lives ranged from minutes at pH 6 to 1 hour at pH 8, for residual chlorine concentrations of 0.5 to 1 mg per L (Acero et al., 2005). The rate of oxidation is pH dependent because hypochlorous acid molecules are primarily responsible for oxidation of MCs, and these molecules rapidly dissociate to hypochlorite ions when pH is greater than 5 (Lawton and Peterson, 1999). Nicholson et al. (1994) observed that aqueous chlorine was more effective than calcium hypochlorite or sodium hypochlorite at the same chlorine concentrations and contact times, and hypothesized the difference in rates was due to sodium hypochlorite and calcium hypochlorite creating more alkaline conditions. Given an initial chlorine concentration of 15 mg per L and a contact duration of 30 minutes, 100% of aqueous MCs (from initial concentrations of 130 to 300 µg per L) were removed for pH values up to 9 for aqueous chlorine disinfectant (Nicholson et al., 1994).

Several byproducts have been characterized following oxidation with chlorine, indicating that MCs are not eliminated from water; rather, they are transformed into various other compounds (Merel et al., 2009). However, Tsuji et al. (1997) observed that byproducts do not elicit protein phosphatase inhibition in the same manner that parent MC compounds do. Although there is no evidence of toxicity from chlorinated MC byproducts, MCs as well as other sources of natural organic matter (NOM) in the water can react with chlorine to form trihalomethanes (THMs), which should be considered if chlorination is selected for removal of aqueous MCs (Svrcek and Smith, 2004; Sharma et al., 2012). Acero et al. (2008) measured a total THM concentration of 110 µg per L following chlorination of MCs (initial concentration of 3 µg per L) at a pH of 7.3 and a chlorine concentration of 3 mg per L, which, for context, is greater than the drinking water criteria in the US and Europe of 80 and 100 µg per L THMs, respectively.

7.4.6 Oxidation with potassium permanganate as a chemical risk management approach for microcystins

Potassium permanganate is also a common oxidant used in drinking water treatment in the US and Europe (Letterman, 1999). Rositano et al. (1998) measured a 95% decrease from an initial aqueous concentration of MCs of 200 µg per L using an exposure of 1 mg per L potassium permanganate for 30 minutes. Rodriguez et al. (2007) measured a second order removal rate coefficient of 357.2 M per second, and removal rates were independent of pH. Concentrations of NOM correlate with the rate of oxidation of MC-LR with permanganate, since humic acids are rapidly oxidized and thus scavenge oxidative potential for MCs. For example, when NOM concentrations were increased from 6 to 10 mg per L, removal efficiency for MCs decreased from 100 to 40%; however, no confounding effects were observed for ammonia or bromide (Acero et al., 2008). Therefore, an important site-specific consideration in estimations of permanganate concentrations necessary would be the concentration and composition of NOM in raw or source water. Potassium permanganate has a slightly higher oxidation potential than chlorine (Lawton and Robertson, 1999), which is likely why increased rate coefficients have been measured at neutral pH as compared to chlorine. However, this process must occur prior to filtration and coagulation steps to eliminate magnesium (Acero et al., 2008); therefore, the concentration achieved must be sufficient to oxidize aqueous MCs but not lyse cyanobacteria cells, which could be challenging to achieve.

7.4.7 Oxidation with hydrogen peroxide as a chemical risk management approach for microcystins

Oxidation of MCs with hydrogen peroxide has had limited or no effectiveness in prior studies. For example, Drikas (1994) reported that 17% removal of MCs was measured for a 60-minute contact duration with 20 mg per L hydrogen peroxide. Rositano et al. (1998) measured no removal of 1 mg per L MC-LR using 2 mg per L of peroxide after 10 minutes. Fawell et al. (1993) also reported that hydrogen peroxide alone was ineffective at removing MCs from both raw and clarified waters. Although hydrogen peroxide alone has not been effective, hydrogen peroxide in combination with UV light and ozone has been effective, as will be discussed in subsequent advanced oxidation sections.

7.4.8 Advanced oxidation with ozone as a chemical risk management approach for microcystins

Ozone (O_3) is an unstable gas with a higher oxidation potential than chlorine, potassium permanganate, or hydrogen peroxide (Lawton and Robertson, 1999), and has been more effective for removal of MCs than these oxidants (Rositano et al., 1998). Upon decomposition of ozone, hydroxyl radicals are produced. Therefore, both inorganic ozone molecules and hydroxyl radicals act as oxidants for aqueous MCs during this process (Svrcek and Smith, 2004). Rositano et al. (1998) measured 100% decrease in aqueous concentrations of MC-LR standard (i.e., isolated toxin) from an initial concentration of 166 µg per L using 0.2 mg per L O_3 for an exposure duration of 4 minutes. As with the oxidants discussed earlier, NOM concentrations can negatively affect performance. In water with a dissolved organic carbon (DOC) concentration of 8.5 mg per L, 1 mg per L O_3 was necessary to achieve 100% removal of MCs (initial concentration of 220 µg per L) with an exposure duration of 5 minutes (Rositano et al., 1998). In both cases, exposure duration was likely more than adequate to achieve the described results, since in a follow-up experiment, 99% removal of 1 mg per L MC-LR in distilled water was achieved in 15 seconds. Shawwa and Smith (2001) measured decreases in MC-LR from 500 to less than 1 µg per L at an exposure of 0.2 mg per L O_3 for a duration of 2 minutes in distilled water. Again, concentrations of NOM influenced effectiveness, as an O_3 exposure of 0.7 mg per L was necessary to achieve the same results at a DOC concentration of 3 mg per L, and 1.0 mg per L O_3 was necessary for 80% decline of MC-LR at a DOC concentration of 5 mg per L (Shawwa and Smith, 2001). If pH is greater than 7, rates of oxidation are likely to decline, since the oxidation potential of O_3 is nearly double in acidic conditions, as compared to alkaline conditions (Rositano et al., 1998). The effects of DOC and pH were supported by Rositano et al. (2001) as the rate of reaction was related to the mass of residual O_3 present after a 5-minute contact duration, which is influenced by the pH and DOC content in water.

In general, ozonation is a rapid oxidation process for MCs, with half-lives of seconds to minutes, which are influenced by site-specific characteristics including alkalinity, DOC concentration, and O_3 concentration (Rositano et al., 1998; Rositano et al., 2001; Brooke et al., 2006; Sharma et al., 2012). Due to the interference of organic carbon with this process, it is logical to rely on physical removal processes for cells and use oxidation by O_3 as a polishing step for aqueous MCs, to maintain as much O_3 residual as possible for contact with dissolved MCs (Svrcek and Smith, 2004). For example, Coral et al. (2013) measured a half-live for O_3 decay of 5.7 minutes at pH 8 in the absence of cyanobacteria cells, as compared to a half-life for decay of O_3 of 0.7 minutes at pH 8 in the presence of $2.5x10^5$ cells per mL. Several byproducts have been identified from this process, and the types of byproducts are influenced by the exposure concentration of O_3, where increased O_3 concentrations result in byproducts of lower molecular weights, yet there is no evidence of toxicity associated with these compounds (Miao et al., 2009; Al Momani et al., 2010; Sharma et al., 2012). Some water utilities may find the expense of this approach to be cost-prohibitive, as capital costs are typically in the tens of millions of dollars and yearly operational and maintenance costs can be in the tens to hundreds of thousands (Mundy et al., 2018).

7.4.9 TiO_2 photocatalysis as a chemical risk management approach for microcystins

Photocatalysis using titanium dioxide (TiO_2) has been investigated for removal of aqueous MCs, with measured half-lives on the order of several minutes in laboratory experiments using distilled waters and electrically-sourced UV (Shepard et al., 1998; Lawton et al., 2003; Fotiou et al., 2013). Interpretation of half-lives reported in literature should be made with caution, since comparing times required for removal is not appropriate in this context (UV irradiance influences rates; Malato et al., 2009). DOC and turbidity can also influence rates of photocatalysis (Pelaez et al., 2011). For example, Shepard et al. (1998) used a MC-LR standard in distilled water with a slurry of TiO_2 exposed to eight 30W 254 nm lamps and measured half-lives of less than 5 minutes. In comparison, when lake water was used, the half-life for MC-YR increased to 21.3 minutes under the same experimental conditions. In a subsequent study using an immobilized film of TiO2 in a recirculating system, half-lives for MC-LR were 2.7 minutes in distilled water and 6 minutes in lake water (pH 8; Shepard et al. 2002). Using a fixed-film of TiO_2 and sunlight, Kinley et al. (2018b) measured half-lives of approximately 111 to 138 minutes (or 0.37 to 0.38 MJ per m^2 in terms of cumulative UV irradiance) for photocatalysis of total MCs (as MC-LR equivalents) in pond water with a DOC concentration of 9 mg per L. Increased half-lives relative to other studies were likely due to the use of sunlight as opposed to electric UV lamps (UV constitutes less than 10% of the solar spectrum) and presence of DOC and turbidity as compared to distilled waters. However, in developing countries that often use unfiltered and untreated surface waters for drinking waters (Funari and Testai, 2008), fixed-film solar photocatalysis could provide a low-energy, low-maintenance water treatment approach when combined with some

form of prior sedimentation or filtration to remove turbidity. To date, there are no known pilot-scale or full-scale experiments regarding photocatalysis of MCs in drinking water treatment plants.

7.4.10 UV + H_2O_2 and ozone + H_2O_2 as chemical risk management approaches for microcystins

When H_2O_2 is illuminated with light at wavelengths greater than 370 nm, hydroxyl radicals are produced that are capable of mineralizing a wide range of organics in water (Dainton and Rowbottom, 1953; Legrini et al., 1993). Rates of UV photolysis of MCs increase in the presence of H_2O_2 (Qiao et al., 2005; He et al., 2012). Qiao et al. (2005) measured a half-life of approximately 15 minutes for UV photolysis of MC-RR using a 254 nm lamp emitting 3.66 mW per cm^2, and no measurable degradation of MC-RR using a concentration of 59.8 mg per L H_2O_2 in darkness. When an irradiance of 3.66 mW per cm^2 was combined with 34 mg per L H_2O_2, the half-life decreased to approximately 10 minutes. pH, initial MC concentration, alkalinity, peroxide concentration, and UV irradiance influenced rates of removal (Qiao et al., 2005); therefore, for site specific trials, feasible rates of removal can be determined in smaller-scale experiments based on resources available. Although rates of removal can increase with increased exposure of H_2O_2, there is a threshold beyond which performance will decline, since H_2O_2 can be oxidized by hydroxyl radicals (Sharma et al., 2012). Qiao et al. (2005) used relatively high UV energy and H_2O_2 concentrations in laboratory studies that may not be feasible for large scale operations. He et al. (2012) found that rate coefficients (in terms of UV irradiance) using 254 nm light emitting 0.27 mW per cm^2 and 30 mg per L H_2O_2 decreased by a factor of 2 to 3 in actual site waters as compared to distilled water, and concluded that water characteristics including NOM and alkalinity could negatively affect performance of this method. Alkalinity can negatively correlate with performance since CO_3^{-2} can scavenge hydroxyl radicals (Sharma et al., 2012). NOM in the water can absorb light in the UV range and can also be oxidized by hydroxyl radicals (Svrcek and Smith, 2004).

Combining ozone (O_3) with H_2O_2 is another approach to effectively oxidize MCs, with effective H_2O_2/O_3 ratios (e.g. mol/mol) for oxidation of organics ranging from 0.3 to 0.6 (Zhou and Smith, 2001). For an initial concentration of 1 mg per L of MC-LR, 0.1 mg per L of H_2O_2 and 0.2 mg per L of O_3 resulted in 100% removal in 30 minutes (Rositano et al., 1998).

7.4.11 Slow sand filtration as a biological risk management approach for microcystins

The goal of slow sand filtration is to achieve physical removal of particulates as well as biological degradation of MCs, due to development of bacterial biofilms on filter surfaces (Svrcek and Smith, 2004). As shown in Table 1, biodegradation half-lives for MCs can range from approximately 1 to 14 days, so it is logical that some level of removal could be expected from biodegradation via slow sand filtration. In a field-scale experiment, Grutzmacher et al. (2002) measured a half-life of 1 hour for dissolved MCs via slow-sand filtration using an average flow rate of 2.5 m^3 per hour and a contact time of 4.5 hours. Comparatively, for filtration of *Planktothrix* at a flow rate of 0.5 m^3 per hour and a contact time of 18 hours, total MC concentrations decreased from approximately 43 to 4 µg per L in 8 days (Grutzmacher et al., 2002). Bacterial density, temperature, and prior exposure of bacterial assemblages to MC-containing algal blooms (i.e., “microcystin memory”) have been suggested as important factors that could influence MC degradation, aerobic or anaerobic (Holst et al., 2003; Chen et al., 2008; Edwards et al. 2008; Chen et al., 2010a). Repeated exposure of filter biofilms to MCs could favor growth and colonization of bacteria capable of degrading MCs, resulting in increased rates of biodegradation. Slow sand filtration is still commonplace in small water treatment systems with part-time operators (Logsdon et al., 2002) and could be useful in areas requiring less energy or a smaller footprint. One consideration is that filtered cyanobacteria cells could senesce and lyse, releasing MCs into the filtrate if not degraded within the filter (Svrcek and Smith, 2004); therefore, low-energy polishing steps (e.g., fixed-film solar photocatalysis) may also be necessary for dissolved MCs. An additional consideration is that, without a prior treatment step, sand filters could rapidly become clogged with cyanobacteria cells (Hendricks, 1991), which would require frequent backwashing.

8 Adaptive water resource management for microcystin-producing cyanobacteria

To this point in this decision support document, data and information have been assembled in a logical order to support our understanding of what microcystins are, how they are structured and can elicit toxicity, how and where they are produced, how people, animals and plants can get exposed, and what types of adverse health effects can and have occurred. Then, high-level comparisons of potential outcomes associated with no-action and action management decisions were made based on the potential exposures and effects that can occur. Following those comparisons, the next section provided pertinent data and information found for available management tactics for "in-lake" control and "in-plant" control. The final segment of this document is designed to provide a template for how to integrate selected tactics and design a management plan for microcystin-producing cyanobacteria. Adaptive water resource management is a process by which the site-specific problem is defined, management goals are clearly stated, and a plan is developed to achieve the stated management goals. Adaptivity is the key word in this process, since uses and functions of water resources change with time, and so can the intensity, frequency, and duration of cyanobacteria blooms from site to site and with time. Thus, careful attention should be paid to how effective and efficient a management plan is working for each site in the event that changes need to be made. It is important to acknowledge that risk management for microcystin-producing cyanobacteria (or any toxin-producing/noxious cyanobacteria) will never be a one-time endeavor and there is no "silver bullet" tactic that will be appropriate for every site. Successful adaptive water resource management in this context would mean control of exposures of microcystin-producing cyanobacteria, microcystins, or both, to ultimately achieve the set management goal.

Adaptive water resource management is a process by which the problem is defined, management goals are clearly stated, and a plan is developed to achieve management goals (Huddleston et al., 2015). Critical to this process is acknowledgement that designated or authorized uses and functions of water resources change with time, as do intensity, spatial distribution, and periodicity of MC-producing cyanobacteria blooms. Therefore, any one of these factors could shift over time, and management plans must be modified accordingly. Further, a certain risk management approach may change in efficacy, durability, or cost effectiveness with time and new approaches may be necessary. Risk management for MC-producers and MCs will not be a one-time endeavor, and water resource managers and decision makers must recognize that persistent efforts and adaptability will be crucial for reliably achieving and sustaining management goals.

In other words, successful and sustained management cannot and will not arise without an effective and adaptable management plan that starts with defining the problem, stating the management goal, and creating the roadmap to achieve that goal.

Problem definition involves characterization of potential or actual exposures and risks at a specific site. This includes determining the source of MC production (e.g., species of MC-producing cyanobacteria), the intensity, spatial distribution, and periodicity of MC-producers. In addition, the magnitude and periodicity of MC production by cyanobacteria should be known (i.e., total MC concentrations, whether production is intermittent or consistent). Then, potential risks that require management are defined, and often these potential risks overlap spatially and temporally with designated uses of or services provided by a water resource. For example, is the water resource used for irrigation, recreation, aquaculture, agriculture, fish and wildlife propagation, drinking water, or combinations of these uses? Based on that information, the exposures that require management can be clearly defined and prioritized.

Once the problem is defined, management goals must be clearly defined, and a specific plan is developed to achieve those management goals. Often, management goals are bound by aspects of time and magnitude (e.g., density of cyanobacteria, total MC concentration). For example, if the management goal is to maintain exposure concentrations of total MCs in drinking water at less than 1 µg per L constantly, then management efforts must be in effect year-round and the target concentration is clear. If the management goal is to maintain recreational use of a water resource, then management is bound by the duration of the recreation season and the recreational guideline or standard values for total MC concentrations.

Successful risk management in this context will be a function of achieving control of MC-producing cyanobacteria and/or MC exposures specifically. To achieve control, water resource managers can review all potential approaches and select one or more that will achieve management goals (Netherland and Schardt, 2012). This decision can be based on relative effectiveness, costs (capital and operational), durability, and availability for each specific site or operation.

In the context of management for drinking water, an integrated risk management plan that incorporates multiple approaches (in-lake and in-plant) has the highest probability of success in achieving management goals. There are several benefits of using source water management as the primary line of defense, with in-plant management as secondary tactics or "polishing steps". First, by controlling densities of MC-producing cyanobacteria within an aquatic system, the source of MC production is being managed. By comparison, when relying solely on in-plant processes for management, the source of production is not being controlled. Rather, exponential increases in concentrations of MCs may enter the plant as cyanobacterial cell densities grow in the water source, requiring rapid and robust treatment processes that can be costly and must be effective in a moment's notice. In this scenario, the treatment plant is the only line of defense for preventing human exposures to MCs.

When early action is taken in a growing season (intervening while cell densities are relatively low), the probability of achieving management goals can increase substantially. Early detection of MC-producing cyanobacteria in a water resource can prompt early action, which starts at the source (i.e., cyanobacteria). Analytical tests for MCs, including enzyme-linked immunosorbent assays (ELISA) and protein phosphatase inhibition assays (PPIA), have relatively low detection limits (e.g., 0.3 µg per L), are widely available, and provide results in hours, which can be useful for screening and early detection of MCs (Hoeger et al., 2005). Once MCs are detected using these assays, there are more sensitive methods available that involve use liquid chromatography with mass spectrometry (LC-MS) (Loftin et al., 2016) for qualitative analysis (i.e., measurements of specific congeners) if that level of resolution is necessary. Since cyanobacteria are the producers of these compounds, analysis of cell densities and identification of species present in the water can be conducted or contracted out to phycology labs for synoptic monitoring. Early detection and early action can minimize cell densities of MC-producing cyanobacteria that require management, which increases the probability of success and decreases costs, since the effectiveness and costs associated with many short-term approaches for MC-producing cyanobacteria are density-dependent. For this reason, it is beneficial to develop a strategic monitoring program at sites that will be managed for MC-producing cyanobacteria in order to trigger (i.e., bring into action) the planned tactics in a proactive manner. Management tactics implemented after visual observation of dense blooms (i.e., reactive management) are more costly, require more persistent effort, and may be less effective or less durable overall.

As previously mentioned, adaptive water resource management involves initial problem definition, stating management goals, and integrated management plan development, but also the ability to learn and adapt plans with time (Huddleston et al., 2015) that may be prompted by changes in water uses, changes in spatial or temporal distribution of MC-producing cyanobacteria, or additional contributions of data to the peer-reviewed literature for human health risks, ecological risks, or risk management approaches. As peer-reviewed case studies regarding successful management plans become available, they will be added to this document.

9 Conclusions

MC-producing cyanobacteria blooms occur rapidly and repeatedly in freshwater resources, creating visible high-pressure situations in which water resource managers must make critical decisions immediately. Peer-reviewed data and other useful information are readily available regarding aspects of MC exposures, potential adverse effects (human health and ecological), and approaches to manage risks. However, the goal of this document was to vet and assembled these data in a manner that aids in logical and efficient decision-making that is based on the best available data. The rationale for assembling information in this document began with the concept that exposures influence risks. Therefore, aspects of MC exposures were characterized in terms of source, structures, environmental and toxicological properties, spatial and temporal distribution, and forms. Next, potential human exposure routes were characterized and ranked in terms of their importance (i.e., routes more likely to result in significant exposures). Based on a strategic literature review with defined data acceptability criteria, data were then compiled for complete exposure pathways for humans. Ecological toxicity data were reviewed to characterize effects thresholds and potencies for mammals, birds, fish, aquatic invertebrates, and plants, and to assemble an SSD based on these data. With exposure and response data summarized, comparisons were made between no-action, exposure avoidance, and control in terms of potential outcomes, with the goal of discerning if risks from no-action decisions were sufficient to warrant risk management. Then, long-term and short-term risk management approaches for MC-producing cyanobacteria and MCs were reviewed in terms of relative effectiveness, availability, durability, and scalability based on peer-reviewed data. Finally, adaptive water resource management was defined and described for this context.

The goal of this decision support document was to provide water resource managers, regulators, and stakeholders with vetted and assembled information to aid in site-specific decision making and development of adaptive water resource management plans. Clearly, there are remaining data gaps (particularly in the area of human health) that require filling, and the information in this document can be adapted as those data become available. We also recognize that there are other well-known toxins produced by cyanobacteria that warrant equal attention and consideration. However, the purpose of this specific document was to highlight a relatively common toxin and to develop a framework for how to analyze and manage risks. Importantly, there is clearly enough information currently available to manage risks associated with MCs effectively and efficiently. With public awareness, stakeholder support, and persistent efforts, unnecessary exposures of humans and other biota to MCs can be avoided, critical uses of freshwater resources can be maintained, and significant financial losses can be prevented.

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Appendix: supplementary tables referenced in text

Table S1. Analytical methods, exposure methods, and measured MC concentrations in vegetables from peer-reviewed literature

Organism	Extraction procedure, analytical method for exposures	Method of exposure to organism	Measured MC Concentration (µg/kg fw)	Reference
Lactuca sativa (lettuce)	Maceration with liquid nitrogen and sonication; ELISA	Irrigation with 100 mL of 0.6-12.5 µg/L once daily for 15d of MCLR and MCRR	8.3-177.8	Hereman & Bittencourt-Oliveira (2012)
Lactuca sativa (lettuce)	Maceration with liquid nitrogen and sonication; ELISA	Irrigation with 2.5-13 µg/L MCLR	39-158	Bittencourt-Oliveira et al. (2016)
Lactuca sativa (lettuce)	Methanol extraction; ELISA and UPLC-MS/MS	Irrigation via spray and drip irrigation in increments of 100 mL, 3x/wk, of 1, 5, and 10 µg/L MCLR	5-75	Lee et al. (2017)
Daucus carota (carrot)	Methanol extraction; ELISA and UPLC-MS/MS	Irrigation via spray and drip irrigation in increments of 100 mL, 3x/wk, of 1, 5, and 10 µg/L MCLR	10-200	Lee et al. (2017)
Phaseolus vulgaris L. (green bean)	Methanol extraction; ELISA and UPLC-MS/MS	Irrigation via spray and drip irrigation in increments of 100 mL, 3x/wk, of 1, 5, and 10 µg/L MCLR	85-88% of total MC mass exposed[a]	Lee et al. (2017)
Lycopersicon esculentum (tomato)	Methanol extraction; LC-MS/MS	Irrigation with 500 mL of 100 µg/L MCLR every third day for 15-d	5.2-10.8[b]	Gutierrez-Praena et al. (2014)

Table S2. Analytical methods and measured MC concentrations in fish and shellfish from peer-reviewed literature. FW = fresh weight

Organism	Extraction procedure, analytical method for exposures	Measured MC Concentration (µg/kg fw)	Reference
Fish			
Tilapia rendalli (redbreast tilapia)	Methanol extraction, ELISA	2-337 (range)	Magalhaes et al. 2001
Oreochromis niloticus (nile tilapia)	Methanol extraction, ELISA	45.7-102 (range)	Mohamed et al. 2003
Cyprinus carpio (common carp)	Methanol extraction, protein phosphatase inhibition assay	38 (mean)	Li et al. 2004
Odontesthes bonariensis (pejerrey)	Methanol extraction, HPLC, LC-MS	50 (mean) 340 (maximum)	Cazenave et al. 2005
Oncorhynchus mykiss (rainbow trout)	Methanol extraction, ELISA	35 (maximum)	Wood et al. 2006
Hypophthalmichthys molitrix (sliver carp)	Methanol extraction, HPLC, LC-MS	124 (mean)	Chen et al. 2007
Shellfish			
Sinanodonta woodiana (Chinese pond mussel)	Butanol/methanol extraction, HPLC-UV	9 (mean) 26 (maximum)	Chen and Xie 2005a
Hyriopsis cumingii (triangle shell mussel)	Butanol/methanol extraction, HPLC-UV	22 (mean) 39 (maximum)	Chen and Xie 2005a
Cristaria plicata (cockscomb pearl mussel)	Butanol/methanol extraction, HPLC-UV	10 (mean) 23 (maximum)	Chen and Xie 2005a
Lamprotula leai (freshwater mussel)	Butanol/methanol extraction, HPLC-UV	21 (mean) 58 (maximum)	Chen and Xie 2005a
Procambarus clarkia (freshwater crayfish)	Butanol/methanol extraction, HPLC-UV	5 (mean) 10 (maximum)	Chen and Xie 2005b
Palaemon modestus (Siberian prawn)	Butanol/methanol extraction, HPLC-UV	6 (mean in muscle) 26 (maximum in muscle) 114 (maximum in whole body)	Chen and Xie 2005b
Macrobrachium nipponensis (freshwater shrimp)	Butanol/methanol extraction, HPLC-UV	4 (mean in muscle) 12 (maximum in muscle) 51 (maximum in whole body)	Chen and Xie 2005b

Table S3. Toxicological data for mammals exposed to MCs. NS= not stated

Organism	Age	Source: Congener(s)	Route	Analytical Method for Exposures	Exposure Duration	Response Measured	Toxicity Value	Citation
Ratus sp. (Sprague-Dawley rat)	NS: Body mass 175-200 g	Purified toxin: LR	IP Injection	HPLC	1.3-d	Mortality	LOEC: 160 µg/kg BW	Hooser et al. 1989
Ratus sp. (rat)	NS	Purified toxin: LR	Oral[a]	HPLC	14-d[b]	Mortality	20% mortality: 5000 µg/kg BW	Fawell et al. 1994
Mus musculus (Swiss albino mouse)	NS: Body mass 18-22 g	Purified extract: LR	IP Injection	LC-MS/MS	1 to 3-h	Mortality	LC50: 35,000 µg/kg BW	Wood et al. 2010a
Mus musculus (mouse)	NS	Purified toxin: LR	Gavage	HPLC	13-wk	Mortality	100% survival (NOEC): 1000 µg/kg BW	Fawell et al. 1994
Mus musculus (mouse)	NS	Purified toxin: LR	IP Injection	HPLC	14-d[b]	Mortality	LC50: 50-158 µg/kg BW	Fawell et al. 1994
Mus musculus (mouse)	NS	Purified toxin: LR	Oral[a]	HPLC	14-d[b]	Mortality	LC50: ~ 5000 µg/kg BW	Fawell et al. 1994

[a] type of oral exposure not specified
[b] surviving animals were monitored for 14-d before euthanization

Table S4. Toxicological data for fish exposed to MCs. NS= not stated

Organism	Age	Source: Congener(s)	Route	Exposure Duration	Analytical Method for Exposures	Response Measured	Toxicity Value	Citation
Oncorhynchus mykiss (rainbow trout)	1-yr	Crude extract: LR[a] Freeze-dried algae: LR[a]	IP injection Gavage	1-d 4-d	HPLC	Mortality Mortality	100% mortality 550 µg/kg-BW[b] 100% mortality 6600 µg/kg-BW[b]	Tencalla et al. 1994
Oncorhynchus mykiss (rainbow trout)	NS: (79-151 g)	Purified extract: LR	IP injection	26-h	HPLC	Mortality	100% mortality 1000 µg/kg-BW[b]	Kotak et al. 1996
Danio rerio (zebrafish)	Embryo	Purified toxin: LR	Direct contact	6-d exposure; responses measured at 21-d	HPLC	Mortality	40% decrease in survival at 5 and 50 µg/L	Oberemm et al. 1997
Danio rerio (zebrafish)	~70-d	Purified toxin: LR	Direct contact[c]	30-d[c]	Plate assay[d]	Body weight	LOEC:1 µg/L	Liu et al. 2014
*Misgurnus mizolepis (*loach*)*	Larvae: newly hatched Juvenile: 30-d	Purified extract: LR	Direct contact	7-d	HPLC	Mortality	7-d LC50=164.3 µg/L (larvae) 593.3 µg/L (30-d juvenile)	Liu et al. 2002
Astyanax bimaculatus (tetra)	NS: 7-10 cm	Crude extract; LR and LA (unspecified % of each)	Direct contact IP injection	3-d	HPLC	Mortality Mortality	72-h LC50=242.8 µg/L 72-h LD50=49.2 µg/kg-BW	Silva et al. 2010

[a] assumed to be LR equivalents; based on LR standard
[b] interpret with caution: only one exposure concentration evaluated
[c] parent generation of 70-d old fish was exposed for 30-d; juveniles produced from parents were never exposed
[d] type of plate assay not specified

Table S5. Toxicological data for aquatic invertebrates exposed to MCs

Organism	Age	Source; Congener(s)	Route	Analytical Method for Exposures	Exposure Duration	Response Measured	Toxicity Value	Citation
Daphnia magna	12±12-h	Purified toxin: LR	Aqueous exposure	HPLC	1-d 2-d 21-d 21-d	Mortality Mortality Mortality Reproduction	24-h LC50=47,000 µg/L 48-h LC50=20,000 µg/ L LOEC=640 µg/L LOEC=360 µg/L	Chen et al. 2005
Daphnia pulicaria	Adult	Purified toxin: LR	Aqueous exposure	HPLC	1-d 2-d	Mortality	24-h LC50 >50,000 µg/L 48-h LC50=21,400 µg/L	DeMott et al. 1991
Daphnia hyalina	Adult	Purified toxin: LR	Aqueous exposure	HPLC	1-d 2-d	Mortality	24-h LC50=34,200 µg/L 48-h LC50=11,600 µg/L	DeMott et al. 1991
Daphnia pulex	Adult	Purified toxin: LR	Aqueous exposure	HPLC	1-d 2-d	Mortality	24-h LC50=10,700 µg/L 48-h LC50=9,600 µg/L	DeMott et al. 1991
Diaptomus birgei	Adult	Purified toxin: LR	Aqueous exposure	HPLC	1-d 2-d	Mortality	24-h LC50=980 µg/L 48-h LC50=450 µg/L	DeMott et al. 1991
Eurytemora affinis	3-4-wk	Purified toxin: LR	Aqueous exposure	HPLC	2-d	Mortality	48-h LC50=270 µg/L	Reinikainen et al. 2002
Tetrahymena pyriformis (ciliate)	300 cells/mL	Purified toxins: LR, LY, LW LF	Aqueous exposure	HPLC	1-d	Growth inhibition	24-h LC50= 252,000 µg/L (LR) 179,000 µg/L (LY) 87,000 µg/L (LW) 83,000 µg/L (LF)	Ward and Codd 1999

Table S6. Toxicological for plants and algae exposed to MCs. NS= not stated

Organism	Age	Source: Congener(s)	Route	Exposure Duration	Analytical Method for Exposures	Response Measured	Toxicity Value (µg/L)	Citation
Lactuca sativa L. (romaine lettuce)	7-wk	Purified toxin: LR	Spray & drip irrigation	4-wk (3 irrigations of 100 mL per wk)	ELISA & UPLC-MS/MS	Length (head of lettuce) Mass (head of lettuce)	LOEC=5 LOEC=5	Lee et al. 2017
Daucus carota (carrots)	7-wk	Purified toxin: LR	Spray & drip irrigation	4-wk (3 irrigations of 100 mL per wk)	ELISA & UPLC-MS/MS	Mass per carrot Diameter of roots	LOEC=1 LOEC=1	Lee et al. 2017
Daucus carota (carrots)	1 to 2-mo	Crude extract: LR (95%); LA (<5%); [D-Asp-3]-LR (<5%)	Watering	28-d (2 irrigations of 40 mL per wk for 4-wk)	ELISA & LC-ESI-MS/MS	Fresh weight per root	NOEC=10 LOEC=50	Machado et al. 2017
Phaseolus vulgaris L. (green beans)	7-wk	Purified toxin: LR	Spray & drip irrigation	4-wk (3 irrigations of 100 mL per week)	ELISA & UPLC-MS/MS	Total mass of beans Number of beans	LOEC=1 LOEC=1	Lee et al. 2017
Oryza sativa (rice)	Seeds	Purified extract: RR (62%); LR (35%); YR (3%)	Aqueous exposure	10-d	ELISA & HPLC	Germination Seedling length Root length[b] Fresh weight roots Dry weight roots	NOEC=3000 LOEC=600 LOEC=120 LOEC=120 LOEC=3000	Chen et al. 2004
Oryza sativa (rice)	Seeds	Crude extract: LR	Aqueous exposure	5-d	HPLC	Length of root crown Crown root number	LOEC=2 LOEC=4	Chen et al. 2012
Brassica napus (rapeseed)	Seeds	Purified extract: RR (62%); LR (35%); YR (3%)	Aqueous exposure	10-d	ELISA & HPLC	Germination Seedling length	LOEC=600 LOEC=120	Chen et al. 2004
Brassica oleracea (broccoli)	47-d post sowing	Purified extract: 3-demethyl-RR (21%) RR (25%) 3-demethyl-LR (21%) LR (33%)	Watering	20-d (once daily watering with unknown volume)	HPLC-PDA (photoiodide array UV detection), LC-ESI-MS, ELISA	Shoot length	NOEC=10	Järvenpää et al. 2007
Sinapis alba (mustard seed)	25-d post sowing	Purified extract: 3-demethyl-RR (21%) RR (25%) 3-demethyl-LR (21%) LR (33%)	Watering	19-d (once daily watering with unknown volume)	HPLC-PDA (photoiodide array UV detection), LC-ESI-MS, ELISA	Shoot length	NOEC=10	Järvenpää et al. 2007
Sinapis alba (mustard seed)	7-d old seedling	Purified toxin: RR	Aqueous exposure	7-d	HPLC	Shoot length	IC_{50}=800	Kurki-Helasmo and Meriluoto 1998
Lemna minor (duckweed)	NS	Purified toxin: LR	Aqueous exposure	5-d	LC-PDA-UV	Average "root" length Longest "root" length Weight Frond number	LOEC=10 LOEC=10 LOEC=10 LOEC=10	Mitrovic et al. 2005
Wolffia arrhiza (rootless uckweed)	NS	Purified toxin: LR	Aqueous exposure	5-d	LC-PDA-UV	Frond number	LOEC=15	Mitrovic et al. 2005
Chladophora fracta (green alga)	40 mg (ww)	Purified toxin: LR	Aqueous exposure	5-d	LC-PDA-UV	Weight	NOEC=10	Mitrovic et al. 2005
Lepidium sativum (watercress)	Seedling	Crude extract: LR	Aqueous exposure	2-d	Protein phosphate inhibition assay	Fresh weight Root length Leaf length	LOEC=1 LOEC=1 LOEC=10	Gehringer et al. 2003

AERF

Aquatic Ecosystem Restoration Foundation

More than twenty years ago, a group of companies formed a nonprofit foundation to address increasing problems with invasive aquatic weeds in complex, multiple-use ecosystems.

The mission of the AERF is to support research and development which provides strategies and techniques for the environmentally and scientifically sound management, conservation and restoration of aquatic ecosystems. Our research provides the basis for the effective control of nuisance and invasive aquatic and wetland plants and algae. Broad strategic goals include:

1. Providing information to the public on the benefits of conserving aquatic ecosystems. This involves various operationally sound methods which are appropriate for a particular water body to achieve the objectives of a sound management plan. This includes the appropriate use of EPA registered aquatic herbicides and algaecides. In addition to **Intervention for microcystin-producing cyanobacteria and microcystins in freshwater resources: Development of a decision support document for risk management**, the Foundation also offers **Biology and Control of Aquatic Plants: A Best Management Practices Manual**, which has become one of the most widely read and used references in the aquatic plant management community. This document can be downloaded from our web site (www.aquatics.org), and illustrates the various ways that aquatic plants can be managed – biological, mechanical, physical, chemical, etc.

2. Providing information and resources to assist regulatory agencies and other entities making decisions that impact aquatic plant management. This goal is partially accomplished by providing independent experts on request to address specifically defined issues. Similarly, AERF has sponsored seminars and symposia throughout the United States on aquatic plant management issues. AERF also assists state and local agencies by providing travel grants for regulatory personnel to participate in aquatic-related professional meetings.

3. Funding research in applied aquatic plant management. AERF has funded ecosystem-related research by independent scientists and graduate students in 20 universities in the United States and with the US Army Corps of Engineers. AERF also promotes the attendance of students at aquatic-related professional meetings by providing assistantships and travel grants to dozens of students annually.

Funding is generated through contributions, sponsorships, donations and grants. The operation of the Foundation is managed by an Executive Director. A Board of Directors, composed of sponsors, provides guidance on the development of annual objectives and the management of fiscal resources. Decisions are made by the Executive Director, such as the selection of subject matter experts, speakers for symposia, AERF participation in seminars and meetings and similar activities that fall within the objectives of the Foundation. A Scientific Advisory Committee composed of PhD researchers comments on the soundness of the science in the research proposals and consistency in terms of the Foundation's mission statement.

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Restoration Regulatory Research

Intervention for microcystin-producing cyanobacteria and microcystins in freshwater resources: Development of a decision support document for risk management

by
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Microcystin (MC) production by cyanobacteria (i.e., blue-green algae) in freshwater resources has resulted in significant financial losses and adverse effects on the health of humans, pets, fish, wildlife, livestock, and plants. As harmful blooms of cyanobacteria (often referred to as harmful algal blooms, or "HABs") increase in frequency, intensity, and severity in freshwater systems throughout the United States and globally, the management decision of "no action" (or a decision not to intervene) results in loss of the beneficial services provided directly and indirectly by the water resource, and increases the likelihood that people and other organisms will be exposed to MCs.

This peer-reviewed publication provides a review of the literature related to risk management of MCs and organizes this information in a logical manner to provide a decision support document for water resource managers, regulators, and stakeholders. We also describe the relative effectiveness, availability, durability, and scalability of long-term and short-term risk management approaches for MC-producing cyanobacteria and MCs based on peer-reviewed data, and we define and describe adaptive water resource management in this context. With public awareness, stakeholder support, and persistent efforts, unnecessary exposures to MCs can be minimized or avoided, critical uses of freshwater resources can be maintained, and significant financial losses can be prevented.

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