Cyanobacterial toxins: Managing human-health and ecological risks from microcystins in surface and drinking waters

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ABSTRACT

Cyanobacteria blooms that produce endotoxins (e.g. microcystins [MCs]) are increasing spatially and temporally in critical freshwater resources worldwide. Due to the potential for human-health and ecological risks from exposures to MCs, designated uses for freshwater resources are often inhibited by MC-producing cyanobacteria blooms. As evidence builds for the risks from MC exposures, as well as financial losses resulting from colonization of MC-producing cyanobacteria in freshwater resources, effective and efficient risk management strategies are urgently needed. The overall goal of this dissertation was to contribute information that supports progress in risk intervention for MC-producing cyanobacteria as well as MCs. To support this goal, the first study involved development of a decision support system for risk management of MC-producing cyanobacteria and MCs. The decision support system included strategic literature reviews with data acceptability criteria, followed by assembly and organization of scientific information necessary to make defensible risk management decisions. Subsequent experiments in this dissertation were conducted to contribute data related to specific risk management approaches for MC-producing cyanobacteria (copper-based algaecide exposures) and MCs (solar fixed-film photocatalysis). For pulse exposures of copper-based algaecides in aquatic systems, cell density is a site specific exposure modifying factor influencing the mass of copper sorbed by the cyanobacteria population and consequent responses. To test this hypothesis, a cyanobacterium, *Microcystis aeruginosa*, was exposed to a copper-based algaecide for a range of cell densities to model the density-dependence of responses in terms of microcystin-LR (MC-LR) release.
in a series of laboratory toxicity experiments. While copper exposure concentrations eliciting comparable extents of MC-LR release ranged an order of magnitude (24-h EC50s 0.03-0.3 mg Cu/L) among cell densities of $10^6$ through $10^7$ cells/mL, copper doses (mg Cu/mg algae) were similar (24-h EC50s 0.005-0.006 mg Cu/mg algae). Knowledge of exposure-response relationships for specific cell densities could refine predictions for in situ exposures and responses, and in turn, decrease the likelihood of amending unnecessary copper concentrations to aquatic systems. In another experiment, hypotheses were tested regarding the fate of MCs following exposures of MC-producing cyanobacteria to a copper-based algaecide. The overall objective was to measure the influence of declining dissolved oxygen (DO) levels (following copper-based algaecide exposures to a range of cyanobacteria cell densities) on resident bacterial assemblages and MC-LR degradation, in mesocosm experiments conducted in a pond in Anderson, SC. DO concentrations had the greatest rate of decline in the highest cell density treatment, followed by medium and low cell densities. MC-LR degradation had half-lives of 1 to 1.9-d among cell densities, with the shortest half-life occurring for the lowest cell density. The relationship between cyanobacteria densities and MC-LR half-lives demonstrated the benefits of mitigating cyanobacteria in early growth stages to minimize MC exposures. In the final experiment, questions were asked regarding an advanced oxidation process that may be useful for altering MC exposures in drinking waters. Following sand filtration of solutions containing a range of cellular: aqueous MC ratios, rates of fixed-film solar photocatalysis using TiO$_2$ were measured for aqueous MCs. Rates and half-lives were calculated as a function of both time and cumulative UV
insolation, to contribute data that would be transferable among seasons and latitudes. In terms of time, half-lives for photocatalysis were approximately 111 to 138-min, and in terms of cumulative UV insolation, half-lives ranged from 0.35 to 0.38 MJ/m². Fixed-film solar photocatalysis achieved half-lives that were less than aerobic and anaerobic biodegradation half-lives reported in peer-reviewed literature (e.g. 1 to 14-d) and could be useful for mitigating risks from MC exposures in drinking water, especially in regions that require low-energy, low-maintenance water treatment methods. This dissertation provided the first known example of a decision support system for intervening in exposures of MC-producing cyanobacteria and MCs in critical freshwater resources. Subsequent experiments contributed data for specific risk management approaches that support early intervention efforts at the source of MC exposures (i.e. cyanobacteria) and rapid transformation of MCs in drinking water using fixed-film solar photocatalysis.
DEDICATION

This dissertation is dedicated to my husband, Howie, who has given me unlimited love and support throughout my graduate school career; to my parents, who taught me responsibility and discipline, and have always been my biggest supporters; and to mine and Howie’s families who have always supported us, even though the distance has been difficult.
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CHAPTER ONE
INTRODUCTION

Endotoxin production by cyanobacteria in freshwater resources has resulted in significant financial losses and adverse effects on the health of humans, domestic animals, fish, wildlife, and plants. Relatively recent 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., 2010; Lurling and Faassen, 2013) illnesses and mortalities following ingestion of cyanobacteria scums or drinking infested water.

Microcystins (MCs) are a prevalent and potent group of toxins produced by cyanobacteria in freshwaters (Funari and Testai, 2008; Cheung et al., 2013; Wood, 2016), for which closures of water resources for drinking water and recreation, as well as human, domestic animal, and wildlife poisonings are frequently attributed.

As MCs in freshwater resources become more prevalent, no decision (or a decision not to intervene) results in loss of services provided directly and indirectly by the water resource, and exposures of people and other organisms to MCs that cannot or do not avoid exposures. In the peer-reviewed literature, data are available 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 specifically, or both. However, these data have not been vetted (i.e.
for quality assurance and quality control) and assembled logically so they can be readily used to make scientifically defensible decisions. The goal of the first chapter in this dissertation was to assemble and organize information necessary to drive risk management for MCs and thereby provide a decision support system for water resource managers, regulators, and interested citizens.

The approach for assembling this information was based on the fundamental toxicological principle that exposures drive risks, and thus, thorough and accurate characterization of exposures is needed to understand how to manage risks. Exposures of MCs are influenced by their source (i.e. cyanobacteria), intrinsic chemical structures and properties, spatial distribution, temporal distribution (i.e. duration and frequency), and form (cell bound or aqueous). Another factor that is crucial in the consequence of exposures is route. Logically, exposures drive responses, and therefore, specific exposure routes and effects, and complete exposure pathways can be parsed for humans and other organisms (mammals, fish, aquatic invertebrates, birds, and plants). Overall, a species sensitivity distribution (SSD) can be assembled for MCs based on toxicological data. Additional information needed to trigger a management decision includes comparisons of the relative risks and outcomes of no action and action. 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. Following these comparisons, specific approaches for risk management were reviewed and compared, based on the management target(s) (i.e. MC-producing cyanobacteria or MCs) to provide information regarding relative effectiveness, scalability, durability, and
availability for different scenarios. Finally, the process of adaptive water resource management was defined and described for this context, and specific examples were provided. Ultimately, the purpose of this decision support system is to provide information necessary to appropriately intervene in MC exposures in a scientifically defensible manner, to be adaptable as more data become available, and to provide site-specificity for a range of situations that are frequently encountered.

Following this initial study, subsequent experiments were conducted to contribute data regarding risk management approaches for both MC-producing cyanobacteria and MCs. Risk management involves altering exposures, since adverse responses are inherently influenced by exposures. Copper-based algaecides have been used for over a century to decrease densities of noxious cyanobacteria and algae (Moore and Kellerman, 1905), which can in turn support management of MC exposures. In order to make predictions of exposures and responses, site-specific factors and processes that could alter exposures of copper-based algaecides should be considered. Intuitively, cyanobacteria cells are not solely the intended receptors for these copper exposures, but are also intrinsic exposure modifying factors. For this reason, the mass of copper sorbed by an algal population is likely driving responses, as opposed to the amended copper exposure concentration alone (De Schamphelaere et al., 2005).

Previous studies have contributed to a widely accepted model that assumes MCs completely release from cyanobacteria cells upon death (Jones and Orr, 1994; WHO, 2011), resulting in restricted uses of copper-based algaecides for cyanobacteria mitigation. Often, studies used to support this “leaky cell hypothesis” have used
unspecified or illegal concentrations of copper algaecides. Recent data to the contrary have shown a relationship between copper exposure concentration and extent of MC release upon cell death (Iwinski et al., 2016a). Since cell densities of cyanobacteria can range orders of magnitude among water resources and within a single water resource over time, amended exposure concentrations of copper resulting in cell membrane lysis and MC release are anticipated to range proportionally. Modeling changes in MC release following copper algaecide exposures for a range of cell densities can provide a metric of the relative change in population-level responses as a function of initial cyanobacterial mass (i.e. ligand availability). Further, sorbed masses of copper eliciting comparable MC release were compared among densities as an additional line of evidence for density dependence.

After questions were resolved regarding density dependence of cyanobacteria responses to copper exposures, the next hypothesis was regarding the fate of MCs following copper algaecide exposures in aquatic systems. There have been concerns about MC persistence in aquatic systems following copper-based algaecide applications based on two perceptions. First, copper could have biocidal effects on bacterial assemblages that are capable of MC biodegradation, which may manifest in decreased rates of MC degradation relative to assemblages not exposed to copper algaecides (Jones and Orr, 1994). The rationale for this perception is that because copper can elicit toxicity to cyanobacteria, then other bacteria assemblages could be adversely affected. Second, if rapid onset of oxygen demand occurs (i.e. due to microbial degradation of cyanobacteria
detritus), density and diversity of bacterial assemblages could be decreased, which could also slow MC degradation rates.

Laboratory and field studies were conducted to evaluate the first perception regarding biocidal properties of copper, and data showed that copper algaecide exposures decreased rates of MC degradation only when copper concentrations were well in excess of legal application concentrations in the US (~ 5 mg Cu/L, where the maximum allowable concentration is 1 mg Cu/L; Iwinski, 2016b; Iwinski et al. 2017). To address the second perception, that rapid DO decline after algaecide exposures could impact bacterial assemblages and consequently MC degradation, a mesocosm-scale study was conducted at the same site with the same copper algaecide used by Iwinski (2016b), without supplying DO. Extent and duration of DO decline following an algaecide exposure are anticipated to correlate with mass of cyanobacteria, since mass of organic matter directly relates to mass of oxygen required for oxidation (Sawyer and McCarty, 1967; Varma and DiGiano, 1968). In addition, total MC concentrations (sum of aqueous and cellular MCs) generally correlate with algal cell densities at a given site (Zohary and Pais Madeira, 1990; Chorus and Bartram, 1999). Given these relationships, it is anticipated that the time necessary to reach non-detect concentrations of MCs following a copper algaecide exposure should decrease with decreasing cell density of cyanobacteria present at the time of exposure, due to less cyanobacteria cells (lesser MC concentrations) and presumably less DO demand (e.g. extent of decline in DO). Rates and extents of total MC-LR degradation can be measured among a range of cyanobacterial densities following copper-based algaecide exposures, with the goal of discerning if there is a
relationship between cell density and DO decline, and ultimately cell density and half-lives for MC-LR degradation.

After questions were asked regarding fate of MC exposures in water resources, a study was conducted to evaluate a process for altering exposures of MCs in drinking water. For humans, one route of exposure can occur from ingestion of aqueous MCs in drinking water, following inability of drinking water treatment processes to remove dissolved MCs from water. Photocatalysis using titanium dioxide (TiO$_2$) has been investigated in prior studies as a potential process for rapid removal of MCs in water, with measured half-lives on the order of minutes (Shepard et al., 1998; Lawton et al., 2003; Fotiou et al., 2013). Previous studies conducted to measure photocatalysis of MCs have used indoor laboratory experimental designs in which confounding factors were minimized (i.e. clear water with high energy UV exposures), offering a “best-case scenario” estimation of half-lives. Design features (e.g. electric UV light or natural sunlight, slurries or fixed-films of catalysts, indoors or outdoors) can be selected based on the specific needs and capabilities of water treatment facilities. However, in the context of developing countries with less access to advanced drinking water treatment methods, low-cost and low-maintenance designs are especially needed. Immobilizing TiO$_2$ on a fixed-film eliminates the need to amend and recover catalyst offering greater flexibility in treatment design. In addition, natural sunlight could provide sufficient energy to accomplish photocatalysis without the use of electric UV light. Sand filtration can physically remove cellular MCs prior to solar photocatalysis, for solutions containing a range of cellular: aqueous MC ratios, to discern the extent of removal possible for
different proportions of cellular and aqueous MCs. In addition, results for a treatment
design with use of sunlight and a fixed-film of TiO$_2$ can be compared with previous
published data to discern the extent to which half-lives can differ under these different
circumstances. For accurate transferability among seasons and latitudes, rates of
photocatalysis can be calculated as a function of cumulative UV insolation.

Organization of Dissertation

This dissertation is arranged in a sequence of chapters intended for publication or
already published in peer-reviewed journals. Therefore, chapters two through five were
written and formatted for specific journals, and some introductory information, and
materials and methods were repeated.

Chapter Two: Intervention for microcystin-producing cyanobacteria and
microcysts in freshwater resources: Development of a decision support system for
risk management

Target journal: *Environmental Monitoring and Assessment*

The overall objective of this study was to assemble and organize information
necessary to drive risk management for MCs, as a decision support system. Specific
objectives were to 1) characterize exposures of MCs (in terms of source, chemical
structures, and environmental fate and toxicological properties, spatial and temporal
distribution, and 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 risk management approaches for cyanobacteria that produce MCs
and MCs, and 5) define and provide examples of adaptive water resource management in the context of risk intervention for MCs.

Chapter Three: Cell density dependence of *Microcystis aeruginosa* responses to copper algaecide concentrations: implications for microcystin-LR release

Published in: *Ecotoxicology and Environmental Safety*

The objectives of this study were to: 1) measure relationships among copper (as copper ethanolamine) exposures and doses for a range of cell densities of *M. aeruginosa* (1x10^6 through 1x10^7 cells/mL), 2) measure responses of *M. aeruginosa* to copper exposures in terms of percent MC-LR release and chlorophyll-a concentrations for each cell density, 3) measure relationships among copper dose and MC-LR release for each cell density, and 4) compare EC50s for MC-LR release in terms of copper exposures and doses among experimental cell densities.

Chapter Four: Microcystin-LR degradation following copper-based algaecide exposures

Published in: *Water, Air, and Soil Pollution*

The overall objective of this study was to measure rates and extents of total MC-LR degradation following exposures of a range of cyanobacterial densities to a copper-based algaecide. Specific objectives were to 1) measure responses in terms of cell density of a cyanobacterial assemblage (*Microcystis aeruginosa, Planktothrix agardhii*, and *Dolichospermum sp.*) in mesocosms with a range of initial cell densities (1x10^6, 5x10^6, and 1x10^7 cells mL^-1) to 96-h exposures of copper (as copper ethanolamine), 2) measure changes in DO concentrations with time after copper exposures for each cell density, 3) measure and compare rates and extents of MC-LR degradation following
copper exposures for each cell density, 4) measure and compare density, diversity, and relative abundance in microbial assemblages among experimental treatments, and 5) compare and contrast data from this study with data for degradation under a range of DO concentrations (with and without copper algaecide exposures) from previous studies.

Chapter Five: Solar photocatalysis using fixed-film TiO$_2$ for microcystins from colonial *Microcystis aeruginosa*

Published in: *Water, Air, and Soil Pollution*

The overall objective of this study was to measure rates of solar photocatalysis of MCs using bench-scale reactors with fixed-films of TiO$_2$ following sand filtration. Specific objectives were to 1) measure and compare extent of total MC removal for sand filtration of a range of cellular: aqueous MC ratios (< 10% aqueous MC, ~50% aqueous MC, and > 90% aqueous MC), 2) using filtered site waters, measure rates and half-lives of solar photocatalysis as a function of cumulative UV insolation and time in fixed-film TiO$_2$ reactors, and 3) contrast measured half-lives with half-lives reported in previous studies using different design features (i.e. slurries of catalyst, different UV sources).
References


CHAPTER TWO

INTERVENTION FOR MICROCYSTIN-PRODUCING CYANOBACTERIA AND MICROCYSTINS IN FRESHWATER RESOURCES: DEVELOPMENT OF A DECISION SUPPORT SYSTEM FOR RISK MANAGEMENT

Abstract

Endotoxin (e.g. microcystin [MC]) production by cyanobacteria in freshwater resources has resulted in significant financial losses and adverse effects on the health of humans, domestic animals, fish, wildlife, and plants. As exposures to MCs in freshwater resources become more frequent, no decision (or a decision not to intervene) results in loss of services provided directly and indirectly by the water resource, and exposures to MCs of people and other organisms that cannot or do not avoid exposures. Peer-reviewed data are available regarding exposures to MCs and effects (human-health and ecological risks), as well as risk management methods for MC-producing cyanobacteria and MCs. However, these data have not been vetted and assembled logically so they can be readily used to make scientifically defensible decisions. The goal of this paper was to assemble and organize the information necessary for risk management of MCs and thereby provide a decision support system for water resource managers, regulators, and interested citizens and stakeholders. The rationale for the objectives in this study was based on the logic that exposures influence risks. Therefore, properties of MC exposures were characterized in terms of source, chemical structures, environmental and toxicological properties, spatial and temporal distribution, and forms (cellular and aqueous). Then, 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 compiled for complete exposure pathways for humans. Ecological toxicity data were then reviewed to characterize effects thresholds and potencies for mammals, birds, fish, aquatic invertebrates, and plants, and to assemble a species sensitivity distribution (SSD) based on these data. Following synthesis of exposure and response data, comparisons were made between no-action, exposure avoidance, and control in terms of potential outcomes, with the goal of evaluating whether risks from no-action decisions were sufficient to warrant risk management. Based on those comparisons, the potential risks of adverse effects and financial losses associated with no-action were clearly sufficient to warrant risk management for MC-producing cyanobacteria (in-lake) as well as MCs in drinking water treatment facilities (in-plant). 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 for this context, and specific examples were provided. There are remaining data gaps, particularly in the area of human health, and the information in this document can be updated as those data become available. However, there is clearly sufficient information available to manage risks effectively and efficiently. With public awareness, stakeholder support, and persistent efforts, unnecessary exposures of humans and other biota to MCs can be minimized or avoided, critical uses of freshwater resources can be maintained, and significant financial losses can be prevented.
Introduction

Endotoxin production by cyanobacteria in freshwater resources has resulted in significant financial losses and adverse effects on the health of humans, domestic animals, fish, wildlife, and plants. Relatively recent 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) are a prevalent and potent group of toxins produced by cyanobacteria in freshwaters (Funari and Testai, 2008; Cheung et al., 2013; Wood, 2016), for which closures of water resources for drinking water and recreation, as well as human, domestic animal, and wildlife poisonings are frequently attributed. Upon advent of a cyanobacterial bloom and consequent public alarm, a common decision by water resource managers is to prevent public use of the water resource, likely due to a lack of scientific information available, experience 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 directly and indirectly by the water resource, and exposures of people and other organisms to MCs that cannot or do not avoid exposures (e.g. Toledo, OH). In the peer-reviewed literature, data are
available 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 and MCs. However, these data have not been vetted (i.e. for quality assurance and quality control) and assembled logically so they can be readily used to assist in making a scientifically defensible decision. The goal of this paper was to assemble and organize the information necessary for risk management of MCs and thereby provide a decision support system for water resource managers, regulators, and interested citizens and stakeholders.

The approach for assembling this information was based on the fundamental toxicological principle that exposures drive risks, and thus, thorough and accurate characterization of exposures is needed first and foremost (Figure 2-1). Exposures of MCs are influenced by their source (i.e. cyanobacteria), their intrinsic chemical structures and properties, spatial distribution, temporal distribution (i.e. duration and frequency), and form (cellular or aqueous). Another factor that is crucial in the consequence of exposures is route of exposure. Logically, exposures also drive responses, and therefore, specific exposure routes and effects can be parsed among humans and other organisms (mammals, fish, aquatic invertebrates, birds, and plants) (Figure 2-1). 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 for different routes.

Regarding ecological risks of MCs, exposure-response relationships for individual species of animals and plants can be evaluated for effects thresholds and potency slopes, which would be necessary especially if these species are keystone or endangered species. Overall, a species sensitivity distribution (SSD) can be assembled for MCs based on toxicological data for animals and plants. Species sensitivity distributions 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. what is 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 2-1). 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, specific approaches for risk management were reviewed and compared, based on management target or goals (i.e. cyanobacteria that produce MCs or MCs) to provide information on relative effectiveness for different scenarios. Finally, the process of adaptive water
resource management was defined for this context, and specific examples were provided. Ultimately, the purpose of this decision support system is to provide 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 system. Specific objectives were to 1) characterize exposures of MCs (in terms of source, chemical structures, and 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 risk management approaches for cyanobacteria that produce MCs and MCs, and 5) define and provide examples of adaptive water resource management in the context of risk intervention for MC-producing cyanobacteria and MCs.
Materials and Methods

Exposures of MCs

Peer-reviewed articles in scientific journals and books, and 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.

Potential human exposure routes

Human exposure routes are parsed as direct and indirect exposures in this paper. Direct exposures were defined as 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 included in this paper were a result of strategic literature reviews based on criteria for primary or secondary evidence. For plant-based food exposures (e.g. vegetables, fruit, grains), studies that reported MC concentrations in

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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 known exposure concentrations from irrigation waters were unavailable, 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 laboratory studies, aquatic organisms have frequently been exposed to unrealistically high concentrations to elicit adverse responses, or unrealistic exposure routes have been used (i.e. intraperitoneal injections), and thus bioconcentration or bioaccumulation of MCs in these organisms may not be realistic.

Potential daily human exposures to MCs from ingestion of 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/kg bw (WHO, 2003). TDIs were calculated for two age groups, with the first age group ranging from birth to < 6-yr and the second age group ranging from 6-yr to 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 has a cumulative uncertainty factor of 1000, added to an already conservative no observed
adverse effects level (NOAEL) derived from Fawell et al. (1994). Therefore, 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.

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 that 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.
Risk of action vs. risk of no action

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 financial impacts.

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.
Results and Discussion

Characterization of MC exposures

Source of MC production

MCs are secondary metabolites produced intermittently or consistently by several genera of cyanobacteria (e.g. *Anabaena, Anabaenopsis, Aphanizomenon, Aphanocapsa, Cylindrospermopsis, Gleotrichia, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Phormidium, Planktothrix, Pseudanabaena, Synechococcus, Synechocystis*, and *Woronochinia*) 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 taking up phosphorus in concentrations well in excess of environmental requirements, known as luxury consumption (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 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 (0-14.6 ppt for *Anabaena torulosa* (Apte et al., 1987), 0-30 ppt for *Oscillatoria* (Fogg et al., 1973; Bishop and Premakumar, 1992), and 5-30 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 approximately 15°C and greater and adequate exposure to sunlight, but this does not indicate growth is not possible in relatively lower water temperatures or in benthic areas with less light penetration. Efforts to monitor for and detect presence and growth of cyanobacteria can support early warning plans for water resources if those data can be 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). Thus, triggering risk management based on cell density of cyanobacteria alone is misguided and can result in false positives. It was concluded from prior batch culture experiments that MC production is positively correlated with nitrogen and phosphorus concentrations (Sivonen, 1990; Vezie et al., 2002), yet it is more likely that nutrient concentrations correlate with population growth rates, rather than MC concentrations per cell (Sevilla et al., 2010; Neilan et al., 2012). Iron deficiency has also correlated with increased MC production in cells (Sevilla et al., 2008). In a laboratory experiment, MC production per cell (i.e. 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 potential for MC production and provides evidence regarding where (spatially) management efforts need to be focused if MC production is occurring.

*Chemical structures and compositions of microcystins (MCs)*

MCs are cyclic heptapeptides with molecular weights ranging from approximately 800-1000 Da (Botes et al. 1982; Carmichael et al., 1988; Namikoshi et al., 1990), that are hepatotoxic (Carmichael, 1994; Feurstein et al., 2009) and phytotoxic (Bittencourt-
Oliveira et al., 2014; Corbel et al., 2014), and consist of over 100 congeners (Wood, 2016, Chen et al., 2018). MCs contain the general structure cyclo-(D-alanine\textsuperscript{1}-X\textsuperscript{2}-D-MeAsp\textsuperscript{3}-Z\textsuperscript{4}-Adda\textsuperscript{5}-D-glutamate\textsuperscript{6}-Mdha\textsuperscript{7}), where X and Z, in the 2\textsuperscript{nd} and 4\textsuperscript{th} 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).

**Mechanisms of toxicity of MCs**

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 lacking for MCs. Protein phosphatases 1 and 2A are found in plants as well, and prior studies have found similar inhibition in plant cells, likely responsible for phytotoxic properties of MCs (MacKintosh et al., 1990).

Protein kinases and phosphatases also play a role 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).

Spatial distribution of MC-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 percent 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 United States Environmental Protection Agency’s (USEPA) National Lake Assessment of 2007, 1,161 lakes (45% natural; 55% man-made reservoirs) were sampled 1-2 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 shores of water resources 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 (Izaguirre 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), ability to withdraw 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.

*Temporal aspects for MC-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 trigger early growth, whereas physical conditions including minimal vertical or horizontal mixing can sustain growth (Paerl, 2014). Duration of a growing season that can support cyanobacteria growth will range with climate and latitude. For example, blooms can occur year-round in humid, warm areas (e.g. Florida, USA), but only during summer months for areas farther from the equator (e.g. Minnesota, USA). 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 at
unpredictable times, 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).

**Temporal aspects of MCs**

Assuming MCs are in the aqueous phase, duration of MC exposures is a function of various fate properties for MCs (Table 2-1), 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, at environmentally relevant pH values (6-8.5), MC-RR and MC-LR are considered similarly hydrophilic (Lawton et al., 2003, Liang et al., 2011), and 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 2-1). 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 of copper-based algaecides (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 < 3-d for aerobic conditions (Iwinski et al., 2016a; Iwinski et al., 2017) and at dissolved oxygen concentrations < 2 mg/L (Kinley et al., 2018a).

Several countries with drinking water guidelines or standards for MCs base final concentration limits on the World Health Organization’s (WHO) provisional guideline value of 1 µg/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/kg body weight (BW) 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 < 6 years old) and adults (≥6 years old through adult) that range from 0.3 to 1.6 µg/L (USEPA, 2015). Some guidelines/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). It is important to recognize that health advisory guidelines (e.g. in the US) are not federally promulgated, thus majority of public water systems are not required to monitor for MC-producing cyanobacteria or MCs in raw or treated waters (unless required by the state, as in Ohio). In a survey conducted by the Association of State Drinking Water Administrations (ASDWA), the majority of states in the US 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.

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.
Exposure routes and effects for humans exposed to MCs

MC exposure routes for humans

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 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 2-2). 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 2-2).

MC exposure via ingestion of drinking water

In drinking waters, exposures are a function of the total MC concentrations 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 exposure concentrations in treated drinking waters have ranged from 0.1 to 12.5 µg/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-detect 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 year olds, and 0.01 to 13.1 µg MC for 6 year olds through adults (Table 2-2). Again, in the majority of situations, MC concentrations in final treated drinking waters are non-detect (i.e. < 0.1 µg/L), but the purpose for this calculation is to estimate potential exposures for humans that could occur from MC concentrations measured in drinking waters.

*MC exposure via 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 bloom to individuals accessing the water resource for recreation. Total MC concentrations (sum of cellular and aqueous) 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/L (Graham et al., 2010; Heiskary et al., 2014; Loftin et al., 2016; Howard et al., 2017; USEPA, 2018). Considering non-adults (e.g. <17 years old) consume on average 49 mL/h when swimming and adults (≥17 years old) consume an average of 21 mL/h (Dufour et al., 2017), possible exposures based on maximum measured MC concentrations could range from 9.3 to 1,790 µg for non-adults and 4 to 767.5 µg for adults for 1-h of swimming (Table 2-2).
MC exposure via 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/kg fresh weight (fw), following irrigation with MC-laden water containing concentrations from 1 to 13 µg/L (Table 2-3). Similarly, MC concentrations in carrots ranged from 10 to 200 µg/kg fw (Table 2-3). Green and ripe tomatoes contained approximately 5 to 11 µg/kg fw following irrigation with 100 µg/L (Table 2-3). Secondary evidence included measured MCs (without knowledge of initial exposure) in lettuce and arugula leaves of 4.7 to 400 µg/kg fw (Codd et al., 1999b; Mohamed and Shehri, 2009) and up to 1200 µg/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-d. Roots of broccoli plants contained 0.9-2.4 µg/kg fw and mustard roots contained 2.5-2.6 µg/kg fw (Jarvenpaa et al., 2007). Chen et al. (2010b) measured MCLR concentrations in apple tree shoots (Malus pumila) of approximately 16, 28, and 225 µg/kg fw following aqueous exposures of 30, 300 and 3000 µg/L for 7-d. Based on average daily intake of vegetables for infants through 5 year olds, exposures of MCs from lettuce, carrots, and tomatoes could range from 0.63-22.5, 1.3-25.3, and 0.63-1.4 µg, respectively (Table 2-2). For adults, exposures could range from 1.5-52.7, 2.9-59.2, and 1.5-3.3 µg, respectively, for lettuce, carrots, and tomatoes (Table 2-2).
**MC exposure via ingestion of grains**

Similar to vegetables and fruits, MC exposures in grains are a function of periodicity of blooms, proximity of bloom to intake for retrieval of waters for irrigation, the initial exposure concentration in irrigation water, and water uptake rates by plants. Studies available for grain exposures met criteria for secondary evidence. Chen et al. (2004) exposed rice (*Oryza sativa*) to aqueous exposures of MCs from 24 to 300 µg/L for 10-d. In rice seedlings, average MC concentrations ranged from 2.94 µg/kg fw in the aqueous exposure of 120 µg/L to 5.4 µg/kg fw in the aqueous exposure of 3000 µg/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). MC-LR was detected in 21 of 44 samples, with a range of concentrations of 0.04 to 3.19 µg/kg dw (Chen et al., 2012). Based on these limited data constituting secondary evidence, MC exposures from rice could range from 0.005-0.38 µg for infants through < 6-yr olds, and from 0.02-1.1 µg for 6-yr olds through adults (Table 2-2).

**MC exposure via 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 have ranged from 1.6 to 100 µg/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/kg fw (Chen et al., 2007) (Table 2-4). These measured MC concentrations are based on methanol or methanol-butanol extractions, which have routinely underestimated the 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 0.007-2.9 µg for children from birth through <6-y old and 0.014-5.4 µg for 6-y old children through adults (Table 2-2). 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.

MC exposure via 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 20-130 µg/kg fw (Table 2-4). Chen and Xie (2005b) measured an average of 5 µg/kg fw MCs in muscle of the crayfish, *Procambarus clarkia* (calculated based on dry
weight concentration and estimated percent moisture content of 90%; Ibelings and Chorus, 2007). Additionally, Chen and Xie (2005b) measured mean MC concentrations in muscle tissue of the shrimp, *Palaemon modestus* and *Macrobrachium nipponensis* of 6 and 4 µg/kg fw, respectively, and maximum muscle tissue concentrations of 26 and 12 µg MC/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/kg fw for *P. modestus* and *M. nipponensis*, respectively (Table 2-4). 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 0.07-2.4 µg for children from birth through <6-y old and 0.2-7.5 µg for 6-y old children through adults (Table 2-2).

*MC exposure via 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). These products are made in 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/g BGAS, with the exception of an outlier (35 µg/g; Dietrich and Hoeger, 2005). BGAS are consumed in doses of 1-4 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/g BGAS, daily exposures could range from 0.4-43.2 µg (Table 2-2). An important consideration for BGAS is that they can be consumed year-round, which would result in a 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).

MC exposure via inhalation

Inhalation of MCs may occur via steam from showering and bathing, or from aerosols during water sports (e.g. boating and jet skiing), and during 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 bath water steam. In terms of aerosolized MC exposures, Backer et al. (2010) measured up to 0.052 ng/m$^3$ of total MCs in air, when total MC concentrations in water ranged from 14.5 – 357 µg/L in two California lakes. In a New Zealand lake containing a bloom of *Microcystis, Anabaena,* and *Aphanathece,* and a maximum measured total MC concentration of 2140 µg/L in the water, maximum measured MC in the air was 0.0018 ng/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 0.0008-0.02 ng for children from birth through <6-y old and 0.001-0.035 ng for 6-y old children through adults for one hour of recreation (Table 2-2). 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 could be of greater concern.

*MC exposure via dermal contact*

Contact with cyanobacterial 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
in skin cells, adverse responses from contact with cyanobacteria cells are acknowledged for human health risks. Reported effects for individuals following contact with cyanobacteria cells producing MCs are discussed in the subsequent effects section of this paper.

*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, 3-4x/wk [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 of 1996 (time of suspected exposure), 101 experienced acute liver injury, and within 7 months, 50 patients died (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 could result in total daily intakes that exceed the TDI recommended by the WHO (Table 2-2). 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 2-2). 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 of an exposure than ingestion of water during swimming, ingestion of vegetables, and ingestion of BGAS based on available data, but still clearly warrant additional data. 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.

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 dosed (e.g. µg MC/kg bw), 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 section of this paper.
Following evaluation of data available from laboratory toxicity experiments, reported human effects following probable exposures (i.e. epidemiological data) were reviewed.

Effects resulting from ingestion of MCs

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 in target organs (i.e. liver, kidney) or based on body weight. Falconer et al. (1994) reported a lowest observed effect concentration (LOEC) of 280 µg/kg bw/d for pigs (44-d duration) based on nominal exposure concentrations (i.e. not measured), while Heinze (1999) reported a LOEC of 50 µg/kg bw/d for mice (28-d 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/kg BW/d for mice in 13-wk 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 we can conclude potential for risk based only on
measured exposures. To predict effects based on measured exposures in food sources or supplements, toxicity experiments would incorporate measured exposures, resulting measured doses (and relative partitioning to organism from food or supplement source), and measured responses. Lack of studies meeting 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 contribution of defensible data (i.e. meeting acceptability criteria; e.g. USEPA, 2002), uncertainty factors in regulatory standards and guidelines could be decreased, increasing our ability to accurately predict the potential for adverse human health effects.

*Effects resulting from inhalation of MCs*

Benson et al. (2005) exposed 6-8-wk old mice to 260 µg MC/m$^3$ (260 ng MC/L) for intervals of 30, 60 and 120 minutes per day for 7-d. Based on estimated aspiration rates of mice, the estimated MC doses were 3, 6, and 12.5 µg/kg/d (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 did observe lesions in the nasal cavities of mice exposed to 6 and 12.5 µg/kg/d, 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/kg/day via inhalation for 7 consecutive days. It should be noted that exposure concentrations used in both Fitzgeorge et al. (1994) and Benson et al. (2005) are 7-8 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.

**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, which include vomiting, diarrhea, nausea (i.e. gastroenteritis), while other symptoms can include skin rashes, eye irritation, and ear irritation (presumably from contact with cyanobacteria cells following recreation) (Bell and Codd, 1994; Piloott 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 recreation. Carmichael (2001) acknowledged that the probability of significant human poisonings from MCs is negligible from water, unless a relatively large quantity of cells were 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.

_Literature review of ecotoxicological data for MCs_

_Toxicity data for mammals exposed to MCs_
Mammals can be exposed to MCs via ingestion of contaminated water, ingestion of shoreline deposits of cyanobacteria scums, or ingestion of food items containing MCs (i.e. plants, fish). In prior laboratory studies, rats and mice have been exposed via IP injection and gavage (Table 2-5). Apparent sensitivities have been 30-100x less when 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 data available 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-d (Fawell et al., 1994; Table 2-5). For gavage exposures over the course of 14-d, 20% mortality was observed in male rats at 5000 µg/kg BW, and approximately 50% mortality was observed among male and female mice at 5000 µg/kg (Table 2-5), 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. LC25 or LC50). For gavage exposures over the course of 13-wk, 0% mortality was measured up to 1000 µg/kg in mice, however 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 µg/kg bw to 5000 µg/kg bw 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 autopsies 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/L levels, and often 
livestock do not have an alternate water resource or do not avoid drinking cyanobacteria 
in water.

Toxicity data for fish exposed to MCs

Fish can be exposed to MCs via direct contact or ingestion of algal cells or food 
sources. Passive uptake of MCs across gill membranes is not anticipated given the 
structure of MCs, rather ingestion of MCs or cyanobacteria cells is likely. For loaches 
(Misgurnus mizolepis), 7-d LC50s occurred at approximately 164 and 593 µg/L for larvae
and juveniles, respectively (Liu et al., 2002; Table 2-6). In 72-h exposures, the LC50 for direct contact to tetras (*Astyanax bimaculatus*) was approximately 243 µg/L, while the LD50 for IP injection was an order of magnitude less at 49 µg/L (Silva et al., 2010). In general, LC50s ranged from 164.3 to 593 µg/L for larval through juvenile fish (Figure 2-3; Table 2-6). Zebrafish embryos (*Danio rerio*) were apparently more sensitive, as an aqueous exposure of 5 µg/L resulted in 60% mortality (Figure 2-3; Table 2-6). 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/L, and 100% mortality at approximately 350 µg/L for a 7-d 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, data pertaining to aqueous exposures were included in the SSD (Figure 2-3).

**Toxicity data for aquatic invertebrates exposed to MCs**

Aquatic invertebrates can be exposed to MCs via aqueous exposures or ingestion of algal cells containing MCs. For *Diaptomus birgei*, 24-h and 48-h LC50s were 980 and 450 µg/L, respectively (DeMott et al., 1991; Table 2-7) in direct contact exposures. Sensitivities of three *Daphnia* species were ranked as *D. pulex* > *D. hyalina* > *D. pulicaria*, and LC50s for these daphnids were 1-2 orders of magnitude greater than the LC50 for *D. birgei* (DeMott et al., 1991; Figure 2-3). Chen et al. (2005) measured 24-h and 48-h LC50s for *Daphnia magna* of 47,000 and 20,000 µg/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-d in the same study, the LOEC for *D. magna* (~17% mortality) decreased to 640 µg/L, and the LOEC for reproduction was 360 µg/L (Chen et al., 2005). An LC50 and EC50 could not be calculated for 21-d 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/L MC-LR, 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-h to 48-h, LC50s for *D. magna* decreased by more than half (47,000 vs. 20,000 µg/L) and further decreased to approximately 1000 µg/L by 21-d (Chen et al., 2005). *Daphnia spp.* are less sensitive than fish previously evaluated, given that 7-d LC50s for larval and juvenile loaches (*M. mizolepis*) were < 1000 µg/L (Liu et al., 2002) and a 30-d LOEC for growth of 70-d old zebrafish (*D. rerio*) was approximately 1 µg/L (Liu et al., 2014).

*Toxicity data for birds exposed to MCs*

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/kg liver weight and average CYN concentrations of 148.3 µg/kg 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/g dw of cyanobacteria cells of MCRR, MCYR, and MCLR, 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-mo old quail (average 205 g BW) 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-d) and sub-chronic (30-d) durations. Birds were force fed via crop probe three times daily in volumes of 10 mL each of masses of MCs of 0.045, 0.46, 4.6, and 46.04 µg (n=5 males for each exposure), which based on the average mass of the experimental quail, is comparable to 0.2, 2.2, 22, and 222 µg MC/kg BW (Skocovska et
al., 2007). No measurable adverse effects were observed in terms of mortality or clinical
signs of disease following the 10-d and 30-d experiments (Skocovska et al., 2007). In a
similar experiment, Damkova et al. (2009) exposed 2-mo old Japanese Quail (n=40) in
breeding pairs (n=20 breeding pairs) to MCs via dietary exposure where MCs were
contained within food provided to the birds daily for 8-wk (n=20 breeding pairs of 40
birds not exposed to MCs for untreated controls). Mean daily consumption was 61.6 µg
MCs (26.5 µg MCRR, 7.6 µg MCYR, and 27.4 µg MCLR). Based on a mean body
weight of 85 g for a Northern Bobwhite quail approximately 55-d post hatch (USEPA,
1993), estimated doses would have been 725 µg/kg BW. 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-d after hatching), or 14-d 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 2-3) does not contain
data for avian species.

Toxicity data for plants and algae exposed to MCs

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/L, regarding decreases in growth (e.g. mass, shoot length, root length) relative to untreated controls (Figure 2-3; Table 2-8). 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/L). Outliers included responses of Oryza sativa, and Brassica napus, for which LOECs were 2-3 orders of magnitude greater than the rest of the dataset for plants (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, as opposed to the majority of the other studies reviewed, in which seeds or plants 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 adverse
consequences for higher organisms, either due to indirect exposures to MCs from ingestion of plants or lack of food available due to adverse effects in plants.

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/L (Figure 2-3). 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/L (i.e. to 10 or 100) can impact a relatively large proportion (i.e. 30-40%) of the total distribution (Figure 2-3). 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.

Relative risks of no-action and action management decisions for MC producing cyanobacteria and MCs

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 costs, of each option were characterized and compared. Since no action refers to unabated growth of cyanobacteria and production of MCs, then, in terms of risk, no action can result in human, animal, and plant exposures and 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 2-9). 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/farm animals, lower crop yields, decline in tourism and recreation (swimming, boating, fishing), and necessity of 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 recreation (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 suggested 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 2-9). Enforced exposure avoidance (an enforced closure of a water resource for drinking water or recreation) can result in lesser 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 (Table 2-9). 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).

**Review of risk management approaches for MC-producing cyanobacteria and MCs**

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, it was concluded 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 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 (Huddleston et al., 2016).

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/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/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 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, 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.

*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 defined as the aging of aquatic systems, or nutrient enrichment of water resources 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 basis 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/L as total phosphorus (TP) (Phillips et al., 2005; Fastner et al., 2016; Schindler et al., 2016). 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 total P from runoff, and human-induced changes in the watershed (e.g. land development) can result in unpredictable outcomes.

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 quicker 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/L to 7.6 µg/L, was achieved in approximately 28-y and following a capital investment equivalent to 4 billion US dollars from countries in the watershed, towards improvements in sewage and wastewater treatment facilities (Jochimsen et al., 2013). Decreases in cyanobacterial biomass from approximately 1 to 0.5 mg/L in the lake occurred after approximately 15-y (Jochimsen et al., 2013). In 2 lakes near Berlin (Schlachtensee and Lake Tegel) decreases of 2-3 orders of magnitude in aqueous total P concentrations were achieved within 5-y 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-y after treatment implementation in Schlachtensee, and 8-y 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 70-80 µg/L (between 1963-1967) to 20-30 µg/L (after 1968) following
diversion of sewage effluent that would normally flow into Lake Washington, into Puget Sound. While chlorophyll-a concentrations in the lake appeared to decrease on the same time scale as total P concentrations (e.g. 4-5 years), the proportion of cyanobacteria in total phytoplankton densities remained at 80-100% for 7-y 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-y. 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 2-3 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-y 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 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 of 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). For example, in a prior laboratory experiment, utilization by phytoplankton of particulate P (84% utilized was non-apatite inorganic P) occurred once soluble P decreased to less than 14 µg/L (Santiago and Thomas, 1992).

Data for successful decline in cyanobacteria biomass all pertain to water resources for which 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 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 make management goals challenging or 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 that could hinder success (e.g. sediment P concentrations,
non-point sources).

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.

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

Short-term risk management approaches for MC-producing cyanobacteria are
based on altering 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 are implemented in drinking water treatment facilities (i.e. in-plant) are 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 2-10).

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

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 water (e.g. < 5 m), 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, Lyngbya), since mixing would likely create a continuous flow of aqueous nutrients for those species. Aeration does not alter the trophic state (e.g. decline in 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.

Often, aeration does not result in decreases in total biomass of algae, rather a shift in composition from a majority of cyanobacteria to chlorophytes (i.e. green algae) and diatoms is likely, 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, aeration could be the solution to one problem (e.g. MC-producing cyanobacteria) but the driver for another problem (e.g. taste and odor producers) in waters used for drinking and recreation. The pressure and volume of air required per unit time to destratify a water body depends on the extent 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). In many cases, these systems are run year-round (e.g. in warmer climates), or can be designed to be triggered by intermittent stratification conditions (Burns, 1994).

*Coagulation and settling as a physical risk 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, aggregate and form larger particles than can settle to the sediments (Huh and Ahn, 2017). Coagulants are not directly algicidal, 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 adverse effects to planktonic cyanobacteria may occur due to light limitation. If coagulants are used for their algicidal 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, resulting in potential for anaerobic conditions at the sediment water interface, and subsequent release of P (Paerl et al., 2016).

Aluminum and ferric salts have 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 addition of 5.3 mg/L alum 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 water column), entrapment in flocculated particles, or potential bioavailability of Al as pH decreases during hydrolysis of Al (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 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, and if light is limited, there is potential for rapid senescence and lysis of cells.

_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, as 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 relatively higher frequencies (e.g. 864 kHz). At higher frequencies, Joyce et al. (2010) attributed adverse effects in *M. 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 for 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/mL of *Aphanizomenon* to non-detect levels within approximately 8-d; 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-h 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 at each specific site.
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 gluconate, copper ethanolamine and triethanolamine, and copper ethanolamine and triethanolamine with adjuvents (e.g. d-limonene). Formulations of hydrogen peroxide-based algaecides include sodium carbonate peroxyhydrate (SCP) and hydrogen peroxide with peroxyacetic acid and acetic acid. Endothall is registered as an algaecide in the form of an alkylamine salt. Certain SCP and copper citrate and gluconate 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 algaecide applicators with permits. 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) and availability of licensed applicators for application at the appropriate time.
Copper has been used as an algaecide for over a century (Moore and Kellerman, 1905), and to date, copper has been 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). At most, half of the total surface area of water resources can be treated at one time, and treatments are not to
exceed every 14-d according to copper algaecide labels in the US. 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 7-20 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. For example, Kinley et al. (2017) measured 24-h EC50s in terms of MC-LR release of 0.03, 0.05, and 0.3 mg Cu/L for cell densities of *Microcystis aeruginosa* of 1x10^6, 5x10^6, and 1x10^7 cells/mL, respectively. Further, concentrations of copper algaecides that decrease viability of 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 (i.e. to the algae), exposures can be minimized for non-target organisms. For example, surface broadcast
sprays 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 and acetic 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$_2$O$_2$/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 more sensitive than eukaryotic algae to exposures of the dimethylalkylamine salt of endothall (Ruzycki et al., 1998). For 96-h laboratory
exposures, EC50s in terms of cell density of two strains of Microcystis aeruginosa were approximately 0.07 and 0.11 mg endothall acid equivalents (a.e.)/L, compared to 96-h EC50s of 0.27 and 0.42 mg a.e./L for chlorophytes Chlamydomonas noctigama and Scenedesmus acuminatus, respectively (Ruzycki et al., 1988). In laboratory toxicity experiments with 7-d exposure durations, Spencer et al. (2013) observed decreased growth of the cyanobacterium Nostoc at an exposure concentration of 0.3 mg endothall a.e./L. However, in a field-scale experiment, no measurable adverse responses in Nostoc were observed up to 5 mg endothall a.e./L, which was attributed to 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), therefore, 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, LC50s for several freshwater fish (e.g. rainbow trout, bluegill sunfish, and golden shiner) and an aquatic invertebrate (e.g. Daphnia magna) range from approximately 0.3-1.3 mg endothall a.e./L (Elf Atochem, 1990). Therefore, caution should be taken for exposure concentrations that are used to control cyanobacteria, and algaecide should be applied to the algae and not to the water column, using broadcast sprays for surficial scums or drop hoses for benthic cyanobacteria.
Certain food-grade dyes (e.g. Aquashade™) are registered 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/L or less for control of algae (Madsen et al., 1999). The label of Aquashade™ instructs not to use this product 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 would be appropriate in small ponds and recreational areas that have little or no outflow of water.

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

In the scientific literature, a couple of approaches are noted 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, 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/m³. However, given what is known regarding MC toxicity to fish (Table 2-6), it is likely not logical to use planktivorous fish as herbivores for MC-producing cyanobacteria.

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

Barley straw (*Hordeum vulagare*) has apparent algistatic properties for cyanobacteria (e.g. *Microcystis aeruginosa*) which may be due to polyphenolics with molecular weights ranging from 1000-3000 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 algistatic or algicidal effects against *Microcystis aeruginosa* over a 6 month duration in a field-scale experiment using 6 experimental ponds. In a laboratory experiment, Jelbart (1993) did
not measure adverse effects from rotting straw on an isolated strain of *Microcystis aeruginosa*. Alternatively, Everall and Lees (1996) measured significant decreases in both cyanobacterial dominance and phytoplankton productivity in reservoir trials using 50 g/m³ of barley straw. To date, the specific compounds responsible for algistatic effects and the modes of action are poorly studied and not well understood. Thus, the reliability of outcomes is questionable. Introduction of organic matter into aquatic systems could result in anaerobic conditions (e.g. from 50 g/m³ barley straw 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.

*Short term risk management approaches for MCs in drinking water treatment (in-plant)*

*Coagulation, flocculation, and sedimentation as physical risk management approaches for MCs*

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/mL) using an exposure of 5.8 mg/L of aluminum sulfate for 6-h 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 site.

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).

*Rapid sand filtration as a physical risk management approach for MCs*

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. For example, Hoeger et al. (2004) observed 98-99% removal of cyanobacteria cells via flocculation, however, percentages can be misleading in the context of cell densities, as a 99% decrease from a cell density of 1.5x10^5 cells/mL results in a cell density of 1.5x10^3 cells/mL. Hoeger et al. (2005) noted that remaining cell densities of >3000 cells/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.

**Powdered and granulated activated carbon as physical risk management approaches for MCs**

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 2-50 nm)
with relatively large pore volumes, ideal for large molecules like MCs (800-1000 Da) that will not effectively sorb to microporous carbons (Donati et al., 1994). For PACs, 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 15-100 mg/L have been effective for decreasing MC concentrations to < 1 µg/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 surface charge of MCs at environmentally relevant pH plays a role in sorption effectiveness to activated carbons. For example, at pH 6-8.5, MC-RR carries a net neutral charge, whereas MC-LR carries a net -1 charge, and MC-LY, MC-LW, and MC-LA carry a net -2 charge (Cook and Newcombe, 2002; Campinas and Rosa, 2006), which may explain why MC-RR has frequently been 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-d at activated carbon concentrations ranging from 100-500 mg/L, however, it was noted that PAC concentrations and contact durations actually used in drinking water treatment plants are much less 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. < 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).
UV Photolysis as a physical risk management approach for MCs

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-min for MC-LR exposed to 147 \( \mu \text{W/cm}^2 \) lamp emitting light at 254 nm. When irradiance was increased to 2550 \( \mu \text{W/cm}^2 \), 100% of MC-LR was removed in 10-min (Tsuji et al., 1995). Qiao et al. (2005) measured a half-life of approximately 15-min using a UV lamp emitting 254 nm wavelength light at 3.66 mW/cm\(^2\) and an initial MC-RR concentration of 720 \( \mu \text{g/L} \). To date, there are no pilot or full-scale experiments published 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 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, 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.

Oxidation with chlorine as a chemical risk management approach for MCs

Disinfection of drinking waters using chlorine has been commonplace since the beginning of the 20\(^{th}\) 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 of chlorine depend on pH and chlorine concentration.
(Acero et al., 2005; Daly et al., 2007). For example, Acero et al. (2005) measured 2\textsuperscript{nd} order rate coefficients for total MCs (sum of congeners) of 475 M (mol/L)/s at pH 4.8 and 9.8 M/s at pH 8.8. For oxidation of MC-LR specifically, half-lives were on the order of minutes at pH 6 up to 1-h at pH 8, for residual chlorine concentrations of 0.5-1 mg/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 at pH > 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/L and a contact duration of 30-min, 100\% of aqueous MCs (from initial concentrations of 130-300 µg/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 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/L following chlorination of MCs (initial concentration of 3 µg/L) at a pH of 7.3 and a chlorine concentration of 3 mg/L, which, for context, is greater than the drinking water criteria in the US and Europe of 80 and 100 µg/L THMs, respectively.

*Oxidation with potassium permanganate as a chemical risk management approach for MCs*

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/L using an exposure of 1 mg/L potassium permanganate for 30-min. Rodriguez et al. (2007) measured a second order removal rate coefficient of 357.2 M (mol/L)/s, 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/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.

Oxidation with hydrogen peroxide as a chemical risk management approach for MCs

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-min contact duration with 20 mg/L hydrogen peroxide. Rositano et al. (1998) measured no removal of 1 mg/L MC-LR using 2 mg/L of peroxide after 10-min. 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 have been effective, as will be discussed in subsequent advanced oxidation sections.

Advanced oxidation with ozone as a chemical risk management approach for MCs

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/L using 0.2 mg/L O$_3$ for an exposure duration of 4-min. As for
the oxidants discussed earlier, NOM concentrations can negatively affect performance. In water with a DOC concentration of 8.5 mg/L, 1 mg/L O<sub>3</sub> was necessary to achieve 100% removal of MCs (initial concentration of 220 µg/L) with an exposure duration of 5-min (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/L MC-LR in distilled water was achieved in 15-s. Shawwa and Smith (2001) measured decreases in MC-LR from 500 to < 1 µg/L at an exposure of 0.2 mg/L O<sub>3</sub> for a duration of 2-min in distilled water. Again, concentrations of NOM influenced effectiveness, as an ozone exposure of 0.7 mg/L was necessary to achieve the same results at a DOC concentration of 3 mg/L, and 1.0 mg/L ozone was necessary for 80% decline of MC-LR at a DOC concentration of 5 mg/L (Shawwa and Smith, 2001). Above pH of 7, rates of oxidation are likely to decline, since the oxidation potential of ozone 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 ozone present after 5-min 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 ozone 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 ozone as a polishing step for aqueous MCs, to maintain as much ozone
residual as possible for contact with dissolved MCs (Svrcek and Smith, 2004). For example, Coral et al. (2013) measured a half-life for ozone decay of 5.7-min at pH 8 in the absence of cyanobacteria cells, as compared to a half-life for decay of ozone 0.7-min at pH 8 in the presence of 2.5x10^5 cells/mL. Several byproducts have been identified from this process, and the types of byproducts are influenced by the exposure concentration of ozone, where increased ozone 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).

**TiO\textsubscript{2} photocatalysis as a chemical risk management approach for MCs**

Photocatalysis using titanium dioxide (TiO\textsubscript{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 time is not an adequate comparator 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\textsubscript{2} exposed to 8-30W 254 nm lamps and measured half-lives of < 5-min. Comparatively, with lake water, the half-life for MC-YR increased to 21.3-min under the same experimental conditions. In a subsequent study using an immobilized film of TiO\textsubscript{2} in a recirculating system, half-lives for MC-LR were 2.7-min in distilled water and 6-min in lake water (pH 8; Shepard et al. 2002). Using a fixed-film of TiO\textsubscript{2} and sunlight, Kinley et al. (2018b) measured half-lives
of approximately 111 to 138-min (or 0.37 to 0.38 MJ/m² 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/L. Increased half-lives relative to other studies were likely due to the use of sunlight as opposed to electric UV lamps (UV constitutes < 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 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.

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UV + H_2O_2 \text{ and Ozone + H}_2O_2 \text{ as chemical risk management approaches for MCs}
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When H₂O₂ is illuminated with light at wavelengths < 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 H₂O₂ (Qiao et al., 2005; He et al., 2012). Qiao et al. (2005) measured a half-life of approximately 15-min for UV photolysis of MC-RR using a 254nm lamp emitting 3.66 mW/cm², and no measurable degradation of MC-RR using a concentration of 59.8 mg/L H₂O₂ in darkness. When an irradiance of 3.66 mW/cm² was combined with 34 mg/L H₂O₂, the half-life decreased to approximately 10-min. 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 H₂O₂, there is a threshold beyond which performance will decline, since H₂O₂ can be oxidized by hydroxyl radicals (Sharma et al., 2012). Qiao et al. (2005) used relatively high UV energy and H₂O₂ 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 254nm light emitting 0.27 mW/cm² and 30 mg/L H₂O₂ decreased by a factor of 2-3 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 CO₃²⁻ can scavenger 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 (O₃) with H₂O₂ is another approach to effectively oxidize MCs, with effective H₂O₂/O₃ 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/L of MC-LR, 0.1 mg/L of H₂O₂ and 0.2 mg/L of ozone resulting in 100% removal in 30-min (Rositano et al., 1998).

Slow sand filtration as a biological risk management approach for MCs

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 2-1, biodegradation half-lives for MC-LR can range from approximately 1 to 14-d, 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-h for dissolved MCs via slow-sand filtration using an average flow rate of 2.5 m$^3$/h and a contact time of 4.5-h. Comparatively, for filtration of Planktothrix at a flow rate of 0.5 m$^3$/h and a contact time of 18-h, total MC concentrations decreased from approximately 43 to 4 µg/L in 8-d (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 footprint. One consideration is that filtered cyanobacteria cells could senesce and lyse, releasing MCs into 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.

**Definition and examples of adaptive water resource management**

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., 2016). Critical to this process is acknowledgement that 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.

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, and 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 < 1 µg/L constantly (Table 2-11), 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 (e.g. Table 2-11).

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 this context, an integrated risk management plan that incorporates multiple approaches (in-lake and in-plant) has the highest probability of success (i.e. achieving management goals). Theoretical examples of different situations that could arise in water resources are provided in Table 2-11, and include problem definition, management goals, and integrated management plans. These scenarios (Table 2-11) are examples of plans that could be developed based on defined problems and stated goals, and are not recommendations.

Early detection of MC-producing cyanobacteria in a water resource can prompt early action, which starts at the source (i.e. cyanobacteria). Analytical assays for MCs including enzyme-linked immunosorbent assays (ELISA) and protein phosphatase
inhibition assays (PPIA) have relatively low detection limits (e.g. 0.3 µg/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. Early detection and early action could minimize cell densities of MC-producing cyanobacteria that require management, which could increase probability of success and decreases costs, since the effectiveness and costs associated with many short-term approaches for MC-producing cyanobacteria are density-dependent. Physical, chemical, biological, or multiple approaches selected from available methods can be implemented to manage MC exposures in water resources via management of MC-producing cyanobacteria.

Intervening in MC exposures at the source (i.e. in source water), rather than in drinking water plants alone has several advantages. First, managing exposures in freshwater resources is not just beneficial for drinking water treatment, but also for managing other exposures that occur aside from drinking water (e.g. recreation, irrigation, agriculture, aquaculture, fish and wildlife). Second, risk management in source waters can decrease the potential cell densities of cyanobacteria and MC concentrations that require removal in-plant, increasing confidence in the ability to achieve management goals, decreasing probability of MC “breakthrough” (e.g. aqueous MCs remaining in drinking water after treatment) and decreasing costs associated with drinking water treatment. Then, physical, chemical, biological, or multiple approaches can be
implemented in drinking water treatment plants to act as additional lines of defense for preventing human exposures.

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., 2016) 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.
Conclusions

MC-producing cyanobacteria blooms occur rapidly and repeatedly in freshwater resources, creating 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, it would be useful to have these data vetted and assembled logically such that a decision can be made logically, efficiently, and most importantly, based on the best available data. The rationale for assembling information in this document began with the fundamental principle that exposures influence risks. Therefore, aspects of MC exposures were characterized in terms of source, chemical structures, environmental and toxicological properties, spatial and temporal distribution, and forms (cellular and aqueous). Then, potential human exposure routes were characterized and ranked in terms of their important (i.e. routes more likely to result in significant exposures). Based on a strategic literature review with defined data acceptability criteria, data were compiled for complete exposure pathways for humans. Ecological toxicity data were then 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 in mind, 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. Based on those comparisons, the potential risks and financial losses associated with no-action were
clearly sufficient to warrant risk management for MC-producing cyanobacteria (in-lake) as well as MCs (in-plant). Long-term and short-term risk management approaches for MC-producing cyanobacteria and MCs were then reviewed in terms of relative effectiveness, availability, durability, and scalability based on peer-reviewed data.

Finally, adaptive water resource management was defined for this context, and examples were provided. The goal of this decision support system was to provide water resource managers, regulators, and interested citizens 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, and the information in this document can be adapted as those data become available. Importantly, there is clearly enough information available currently to manage risks 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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Table 2-1. Physical, chemical, and biological characteristics of MC-LR

<table>
<thead>
<tr>
<th><strong>Microcystin-LR (L – leucine, R – arginine)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
</tr>
<tr>
<td><strong>CAS Number</strong></td>
</tr>
<tr>
<td><strong>Color/ Form</strong></td>
</tr>
<tr>
<td><strong>Density</strong></td>
</tr>
<tr>
<td><strong>Photolysis (half-life)</strong></td>
</tr>
<tr>
<td><strong>Photocatalysis (half-life)</strong></td>
</tr>
<tr>
<td><strong>Hydrolysis</strong></td>
</tr>
<tr>
<td><strong>Microbial Aerobic Degradation (half-life)</strong></td>
</tr>
<tr>
<td><strong>Microbial Anaerobic Degradation (half-life)</strong></td>
</tr>
<tr>
<td><strong>Solubility in Water</strong></td>
</tr>
<tr>
<td><strong>Boiling point (°C)</strong></td>
</tr>
<tr>
<td><strong>Log(_{Dow}) (n-octanol/water distribution ratio)</strong></td>
</tr>
<tr>
<td><strong>K(_d) (L kg(^{-1})) (Sediment)</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Exposure Source</th>
<th>Human age (yr)</th>
<th>Daily Ingestion Rate&lt;sup&gt;a&lt;/sup&gt; (g/kg BW/d unless otherwise noted)</th>
<th>Range of [MC] in source (µg/kg fw unless otherwise noted)</th>
<th>Estimated MC Exposure (µg/d) (unless otherwise noted)</th>
<th>Exceed TDI? (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking Water</td>
<td>Birth – 5</td>
<td>0.36 L/d</td>
<td>0.01 – 12.5 µg/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.004 – 4.5</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6- adult</td>
<td>1.05 L/d</td>
<td></td>
<td>0.011 – 13.1</td>
<td>Y</td>
</tr>
<tr>
<td>Water During Swimming</td>
<td>&lt;17</td>
<td>49 mL/h&lt;sup&gt;b&lt;/sup&gt;</td>
<td>189 – 36549 µg/L&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.3 – 1,790</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>≥17</td>
<td>21 mL/h&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>4 – 767.5</td>
<td>Y</td>
</tr>
<tr>
<td>Lettuce*</td>
<td>Birth – 5</td>
<td>6.8</td>
<td>5-178&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.6 – 22.5</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6 - adult</td>
<td>3.7</td>
<td></td>
<td>1.5 – 52.7</td>
<td>Y</td>
</tr>
<tr>
<td>Carrots*</td>
<td>Birth – 5</td>
<td>6.8</td>
<td>10-200&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.3 – 25.3</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6 - adult</td>
<td>3.7</td>
<td></td>
<td>3 – 59.2</td>
<td>Y</td>
</tr>
<tr>
<td>Tomatoes*</td>
<td>Birth – 5</td>
<td>6.8</td>
<td>5-11&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.63 – 1.4</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6 - adult</td>
<td>3.7</td>
<td></td>
<td>1.5 – 3.3</td>
<td>Y</td>
</tr>
<tr>
<td>Rice*</td>
<td>Birth – 5</td>
<td>6.4</td>
<td>0.04-3.19&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.005 – 0.38</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>6 - adult</td>
<td>4.4</td>
<td></td>
<td>0.01 – 1.1</td>
<td>N</td>
</tr>
<tr>
<td>Finfish*</td>
<td>Birth – 5</td>
<td>1.6</td>
<td>0.25-100&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.007 – 2.9</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6 - adult</td>
<td>0.68</td>
<td></td>
<td>0.014 – 5.4</td>
<td>Y</td>
</tr>
<tr>
<td>Shellfish*</td>
<td>Birth – 5</td>
<td>1</td>
<td>4-130&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.07 – 2.4</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6 - adult</td>
<td>0.72</td>
<td></td>
<td>0.2 – 7.5</td>
<td>Y</td>
</tr>
<tr>
<td>Blue-green algal supplements</td>
<td>Birth – 5</td>
<td>4 g/d&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.1-10.8 µg/g&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.4 – 43.8</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>6- adult</td>
<td>6.86 m&lt;sup&gt;3&lt;/sup&gt;/h&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
<td>0.0018-0.052 ng/m&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;j&lt;/sup&gt;</td>
<td>N</td>
</tr>
<tr>
<td>Inhalation:   recreation</td>
<td>Birth - 5</td>
<td>0.42 m&lt;sup&gt;3&lt;/sup&gt;/h&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.0018-0.052 ng/m&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.0008 – 0.02 ng</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>6 - adults</td>
<td>0.68 m&lt;sup&gt;3&lt;/sup&gt;/h&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
<td>0.001 – 0.035 ng</td>
<td>N</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<sup>b</sup> Lahti et al. (2001); Blaha and Marsalek (2003); Hoeger et al. (2004); Jacoby and Kann (2007); Burns (2008)

<sup>c</sup> Graham et al. (2010); Heiskary et al. (2014); Loftin et al. (2016); USEPA (2016); Howard et al. (2017)
\(^4\) Hereman & Bittencourt-Oliveira (2012); Bittencourt-Oliveira et al. (2016); Lee et al. (2017)
\(^5\) Lee et al. (2017); \(^6\) Gutierrez-Praena et al. (2014); \(^7\) Chen et al. (2012)
\(^8\) Mohamed et al. (2003); Li et al. (2004); Cazenave et al. (2005); Shen et al. (2005); Wood et al. (2006)
\(^1\) Chen and Xie (2005a and b)
\(^9\) Gilroy et al., 2000; Lawrence et al., 2001; Hoeger & Dietrich, 2004; Saker et al., 2005; Vichi et al., 2012
\(^a\) Based on values from USEPA (2011) adjusted for 1-h (from values originally based on 24-h)
\(^b\) Backer et al. (2010); Wood and Dietrich (2011)
Table 2-3. Analytical methods, exposure methods, and measured MC concentrations in vegetables from peer-reviewed literature

<table>
<thead>
<tr>
<th>Organism</th>
<th>Extraction procedure, analytical method for exposures</th>
<th>Method of exposure to organism</th>
<th>Measured MC Concentration (µg/kg fw)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactuca sativa</em> (lettuce)</td>
<td>Maceration with liquid nitrogen and sonication; ELISA</td>
<td>Irrigation with 100 mL of 0.6 – 12.5 µg/L once daily for 15-d of MCLR and MCRR</td>
<td>8.3 - 177.8</td>
<td>Hereman &amp; Bittencourt-Oliveira (2012)</td>
</tr>
<tr>
<td><em>Lactuca sativa</em> (lettuce)</td>
<td>Maceration with liquid nitrogen and sonication; ELISA</td>
<td>Irrigation with 2.5 – 13 µg/L MCLR</td>
<td>39 - 158</td>
<td>Bittencourt-Oliveira et al. (2016)</td>
</tr>
<tr>
<td><em>Lactuca sativa</em> (lettuce)</td>
<td>Methanol extraction; ELISA and UPLC-MS/MS</td>
<td>Irrigation via spray and drip irrigation in increments of 100 mL, 3x/wk, of 1, 5, and 10 µg/L MCLR</td>
<td>5 - 75</td>
<td>Lee et al. (2017)</td>
</tr>
<tr>
<td><em>Daucus carota</em> (carrot)</td>
<td>Methanol extraction; ELISA and UPLC-MS/MS</td>
<td>Irrigation via spray and drip irrigation in increments of 100 mL, 3x/wk, of 1, 5, and 10 µg/L MCLR</td>
<td>10 - 200</td>
<td>Lee et al. (2017)</td>
</tr>
<tr>
<td><em>Phaseolus vulgaris L.</em>      (green bean)</td>
<td>Methanol extraction; ELISA and UPLC-MS/MS</td>
<td>Irrigation via spray and drip irrigation in increments of 100 mL, 3x/wk, of 1, 5, and 10 µg/L MCLR</td>
<td>85-88% of total MC mass exposed(^{a})</td>
<td>Lee et al. (2017)</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em>    (tomato)</td>
<td>Methanol extraction; LC-MS/MS</td>
<td>Irrigation with 500 mL of 100 µg/L MCLR every third day for 15-d</td>
<td>5.2 – 10.8(^{b})</td>
<td>Gutierrez-Praena et al. (2014)</td>
</tr>
</tbody>
</table>
Table 2-4. Analytical methods and measured MC concentrations in fish and shellfish from peer-reviewed literature

<table>
<thead>
<tr>
<th>Organism</th>
<th>Extraction procedure, analytical method for exposures</th>
<th>Measured MC Concentration (µg/kg fw)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tilapia rendalli</em> (redbreast tilapia)</td>
<td>Methanol extraction, ELISA</td>
<td>2 – 337 (range)</td>
<td>Magalhaes et al. 2001</td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em> (nile tilapia)</td>
<td>Methanol extraction, ELISA</td>
<td>45.7 - 102 (range)</td>
<td>Mohamed et al. 2003</td>
</tr>
<tr>
<td><em>Cyprinus carpio</em> (common carp)</td>
<td>Methanol extraction, protein phosphatase inhibition assay</td>
<td>38 (mean)</td>
<td>Li et al. 2004</td>
</tr>
<tr>
<td><em>Odontesthes bonariensis</em> (pejerrey)</td>
<td>Methanol extraction, HPLC, LC-MS</td>
<td>50 (mean)</td>
<td>Cazenave et al. 2005</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em> (rainbow trout)</td>
<td>Methanol extraction, ELISA</td>
<td>35 (maximum)</td>
<td>Wood et al. 2006</td>
</tr>
<tr>
<td><em>Hypophthalmichthys molitrix</em> (sliver carp)</td>
<td>Methanol extraction, HPLC, LC-MS</td>
<td>124 (mean)</td>
<td>Chen et al. 2007</td>
</tr>
<tr>
<td><strong>Shellfish</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sinanodonta woodiana</em> (chinese pond mussel)</td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>9 (mean)</td>
<td>Chen and Xie, 2005a</td>
</tr>
<tr>
<td><em>Hyriopsis cumingii</em> (triangle shell mussel)</td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>22 (mean)</td>
<td>Chen and Xie, 2005a</td>
</tr>
<tr>
<td><em>Cristaria plicata</em> (cockscomb pearl mussel)</td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>10 (mean)</td>
<td>Chen and Xie, 2005a</td>
</tr>
<tr>
<td><em>Lamprotula leai</em> (freshwater mussel)</td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>21 (mean)</td>
<td>Chen and Xie, 2005a</td>
</tr>
</tbody>
</table>

133
<table>
<thead>
<tr>
<th>Species</th>
<th>Extraction Method</th>
<th>Concentration (units)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Procambarus clarkia</em></td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>5 (mean) 10 (maximum)</td>
<td>Chen and Xie, 2005b</td>
</tr>
<tr>
<td><em>Palaemon modestus</em></td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>6 (mean in muscle) 26 (max. in muscle) 114 (max in whole body)</td>
<td>Chen and Xie, 2005b</td>
</tr>
<tr>
<td><em>Macrobrachium nipponensis</em></td>
<td>Butanol/methanol extraction, HPLC-UV</td>
<td>4 (mean in muscle) 12 (max. in muscle) 51 (max in whole body)</td>
<td>Chen and Xie, 2005b</td>
</tr>
</tbody>
</table>
Table 2-5. Toxicological data for mammals exposed to MCs. NS= not stated.

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

<sup>a</sup>type of oral exposure not specified  
<sup>b</sup>surviving animals were monitored for 14-d before euthanization
Table 2-6. Toxicological data for fish exposed to MCs. NS= not stated.

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

^aassumed to be LR equivalents; based on LR standard
^binterpret with caution: only one exposure concentration evaluated
^cparent generation of 70-d old fish was exposed for 30-d; juveniles produced from parents were never exposed
^dtype of plate assay not specified
Table 2-7. Toxicological data for aquatic invertebrates exposed to MCs.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Age</th>
<th>Source; Congener(s)</th>
<th>Route</th>
<th>Analytical Method for Exposures</th>
<th>Exposure Duration</th>
<th>Response Measured</th>
<th>Toxicity Value</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia magna</em></td>
<td>12±12-h</td>
<td>Purified toxin: LR</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>1-d</td>
<td>Mortality</td>
<td>24-h LC50= 47,000 µg/L</td>
<td>Chen et al. 2005</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>48-h LC50= 20,000 µg/L</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>2-d</td>
<td>Mortality</td>
<td>LOEC= 640 µg/L</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21-d</td>
<td>Mortality</td>
<td>LOEC= 360 µg/L</td>
<td></td>
</tr>
<tr>
<td><em>Daphnia pulicaria</em></td>
<td>Adult</td>
<td>Purified toxin: LR</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>1-d</td>
<td>Mortality</td>
<td>24-h LC50 &gt;50,000 µg/L</td>
<td>DeMott et al. 1991</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>48-h LC50 = 21,400 µg/L</td>
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</tr>
<tr>
<td><em>Daphnia hyalina</em></td>
<td>Adult</td>
<td>Purified toxin: LR</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>1-d</td>
<td>Mortality</td>
<td>24-h LC50= 34,200 µg/L</td>
<td>DeMott et al. 1991</td>
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<td></td>
<td>48-h LC50= 11,600 µg/L</td>
<td></td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>Adult</td>
<td>Purified toxin: LR</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>1-d</td>
<td>Mortality</td>
<td>24-h LC50= 10,700 µg/L</td>
<td>DeMott et al. 1991</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>48-h LC50= 9,600 µg/L</td>
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<td></td>
<td>2-d</td>
<td>Mortality</td>
<td></td>
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<tr>
<td><strong>Table 2-7. (continued)</strong></td>
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</tr>
<tr>
<td><strong>Diaptomus birgei</strong></td>
<td>Adult</td>
<td>Purified toxin: LR</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>1-d</td>
<td>24-h LC50= 980 µg/L</td>
<td>DeMott et al. 1991</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-d</td>
<td></td>
<td>48-h LC50= 450 µg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Eurytemora affinis</strong></td>
<td>3-4-wk</td>
<td>Purified toxin: LR</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>2-d</td>
<td>Mortality</td>
<td>48-h LC50= 270 µg/L</td>
<td>Reinikainen et al. 2002</td>
</tr>
<tr>
<td><strong>Tetrahymena pyriformis</strong></td>
<td>300 cells/mL</td>
<td>Purified toxins: LR, LY, LW</td>
<td>Aqueous exposure</td>
<td>HPLC</td>
<td>1-d</td>
<td>Growth inhibition</td>
<td>24-h LC50= 252,000 µg/L (LR)</td>
<td></td>
</tr>
<tr>
<td>(ciliate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>179,000 µg/L (LY)</td>
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<td></td>
<td></td>
<td>87,000 µg/L (LW)</td>
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<td></td>
<td>83,000 µg/L (LF)</td>
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</tr>
</tbody>
</table>
Table 2-8. Toxicological for plants and algae exposed to MCs. NS= not stated.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Age</th>
<th>Source: Congener(s)</th>
<th>Route</th>
<th>Exposure Duration</th>
<th>Analytical Method for Exposures</th>
<th>Response Measured</th>
<th>Toxicity Value (µg/L)</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactuca sativa</em> L. (Romaine lettuce)</td>
<td>7-wk</td>
<td>Purified toxin: LR</td>
<td>Spray &amp; drip irrigation</td>
<td>4-wk (3 irrigations of 100 mL per wk)</td>
<td>ELISA &amp; UPLC-MS/MS</td>
<td>Length (head of lettuce) Mass (head of lettuce)</td>
<td>LOEC = 5  LOEC = 5</td>
<td>Lee et al. 2017</td>
</tr>
<tr>
<td><em>Daucus carota</em> (carrots)</td>
<td>7-wk</td>
<td>Purified toxin: LR</td>
<td>Spray &amp; drip irrigation</td>
<td>4-wk (3 irrigations of 100 mL per wk)</td>
<td>ELISA &amp; UPLC-MS/MS</td>
<td>Mass per carrot Diameter of roots</td>
<td>LOEC = 1  LOEC = 1</td>
<td>Lee et al. 2017</td>
</tr>
<tr>
<td><em>Daucus carota</em> (carrots)</td>
<td>1 to 2-mo</td>
<td>Crude extract: LR (95%) LA (&lt;5%) [D-Asp-3]-LR (&lt;5%)</td>
<td>Watering</td>
<td>28-d (2 irrigations of 40 mL per wk for 4-wk)</td>
<td>ELISA &amp; LC-ESI-MS/MS</td>
<td>Fresh weight per root</td>
<td>NOEC = 10 NOEC = 50</td>
<td>Machado et al. 2017</td>
</tr>
<tr>
<td><em>Phaseolus vulgaris</em> L. (green beans)</td>
<td>7-wk</td>
<td>Purified toxin: LR</td>
<td>Spray &amp; drip irrigation</td>
<td>4-wk (3 irrigations of 100 mL per week)</td>
<td>ELISA &amp; UPLC-MS/MS</td>
<td>Total mass of beans Number of beans</td>
<td>LOEC = 1  LOEC = 1</td>
<td>Lee et al. 2017</td>
</tr>
<tr>
<td><em>Oryza sativa</em> (rice)</td>
<td>Seeds</td>
<td>Purified extract: RR (62%) LR (35%) YR (3%)</td>
<td>Aqueous exposure</td>
<td>10-d</td>
<td>ELISA &amp; HPLC</td>
<td>Germination Seedling length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length Root length</td>
<td>NOEC = 3000 NOEC = 600 NOEC = 120 NOEC = 120 NOEC = 3000</td>
<td>Chen et al. 2004</td>
</tr>
<tr>
<td><em>Brassica napus</em> (rapeseed)</td>
<td>Seeds</td>
<td>Purified extract: RR (62%) LR (35%) YR (3%)</td>
<td>Aqueous exposure</td>
<td>10-d</td>
<td>ELISA &amp; HPLC</td>
<td>Germination Seedling length</td>
<td>LOEC = 600 LOEC = 120</td>
<td>Chen et al. 2004</td>
</tr>
<tr>
<td>Plant Species</td>
<td>Treatment Duration</td>
<td>Treatment Method</td>
<td>Watering Details</td>
<td>Analytical Method</td>
<td>Outcome</td>
<td>Reference</td>
<td></td>
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</tr>
<tr>
<td><em>Brassica oleracea</em> (broccoli)</td>
<td>47-d post sowing</td>
<td>Purified extract:</td>
<td>3-demethyl-RR (21%) RR (25%) 3-demethyl-LR (21%) LR (33%)</td>
<td>HPLC-PDA (photoiodide array UV detection), LC-ESI-MS, ELISA</td>
<td>Shoot length NOEC = 10</td>
<td>Järvenpää et al. 2007</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>RR (25%) 3-demethyl-LR (21%) LR (33%)</td>
<td>20-d (once daily watering with unknown volume)</td>
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<tr>
<td><em>Sinapis alba</em> (mustard seed)</td>
<td>25-d post sowing</td>
<td>Purified extract:</td>
<td>3-demethyl-RR (21%) RR (25%) 3-demethyl-LR (21%) LR (33%)</td>
<td>HPLC-PDA (photoiodide array UV detection), LC-ESI-MS, ELISA</td>
<td>Shoot length NOEC = 10</td>
<td>Järvenpää et al. 2007</td>
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<tr>
<td></td>
<td></td>
<td>RR (25%) 3-demethyl-LR (21%) LR (33%)</td>
<td>19-d (once daily watering with unknown volume)</td>
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<tr>
<td><em>Sinapis alba</em> (mustard seed)</td>
<td>7-d old seedling</td>
<td>Purified toxin:</td>
<td>RR</td>
<td>HPLC</td>
<td>Shoot length IC₅₀ = 800</td>
<td>Kurki-Helasmo and Meriluoto 1998</td>
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<tr>
<td></td>
<td></td>
<td>Aqueous exposure</td>
<td>7-d</td>
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<td></td>
</tr>
<tr>
<td><em>Lemna minor</em> (Duckweed)</td>
<td>NS</td>
<td>Purified toxin:</td>
<td>RR</td>
<td>HPLC</td>
<td>Shoot length</td>
<td>Mitrovic et al. 2005</td>
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<tr>
<td></td>
<td></td>
<td>LR</td>
<td>Aqueous exposure 5-d</td>
<td></td>
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<tr>
<td><em>Wolffia arrhiza</em> (Rootless duckweed)</td>
<td>NS</td>
<td>Purified toxin:</td>
<td>LR</td>
<td>HPLC</td>
<td>Frond number LOEC = 15</td>
<td>Mitrovic et al. 2005</td>
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<tr>
<td></td>
<td></td>
<td>LR</td>
<td>Aqueous exposure 5-d</td>
<td></td>
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</tr>
<tr>
<td><em>Chladophora fracta</em> (green alga)</td>
<td>40 mg (ww)</td>
<td>Purified toxin:</td>
<td>LR</td>
<td>HPLC</td>
<td>Weight NOEC = 10</td>
<td>Mitrovic et al. 2005</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>LR</td>
<td>Aqueous exposure 5-d</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Lepidium sativum</em> (Watercress)</td>
<td>Seedling</td>
<td>Crude extract:</td>
<td>LR</td>
<td>Protein phosphate inhibition assay</td>
<td>Fresh weight LOEC = 1</td>
<td>Gehringer et al. 2003</td>
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<tr>
<td></td>
<td></td>
<td>Aqueous exposure</td>
<td>2-d</td>
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<td>Root length</td>
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<td>Leaf length</td>
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</table>

**Table 2-8. (continued)**
Table 2-9. Potential outcomes associated with no-action, exposure avoidance, and control decisions

<table>
<thead>
<tr>
<th>Risk Management Decision</th>
<th>Potential Outcomes</th>
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</thead>
<tbody>
<tr>
<td><strong>No Action</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unrestricted human exposures: gastrointestinal symptoms, skin rashes and irritation, eye and ear irritation, respiratory symptoms, liver failure</td>
</tr>
<tr>
<td></td>
<td>Unrestricted livestock and pet exposures: gastrointestinal symptoms, weakness, liver failure, death, financial and personal losses</td>
</tr>
<tr>
<td></td>
<td>Unrestricted ecological exposures: fish kills, bird mortalities, plant (crop) mortalities, risks to endangered and keystone species, financial losses</td>
</tr>
<tr>
<td></td>
<td>Loss of designated uses of water: inability to provide services, financial losses</td>
</tr>
<tr>
<td><strong>Exposure Avoidance</strong></td>
<td></td>
</tr>
<tr>
<td>Unenforced</td>
<td>Potential for human and pet exposures: see effects above</td>
</tr>
<tr>
<td></td>
<td>Unrestricted livestock and ecological exposures: see effects and financial losses above</td>
</tr>
<tr>
<td>Enforced</td>
<td>Unrestricted livestock and ecological exposures: see effects above</td>
</tr>
<tr>
<td></td>
<td>Loss of designated uses of water: see financial losses above</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Avoidable human exposures prevented</td>
</tr>
<tr>
<td></td>
<td>Avoidable domestic pet and livestock exposures prevented</td>
</tr>
<tr>
<td></td>
<td>Avoidable ecological exposures prevented</td>
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<tr>
<td></td>
<td>Designated uses of water resource maintained or regained</td>
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<td></td>
<td>Costs associated with control technique(s)</td>
</tr>
<tr>
<td></td>
<td>Collateral damage associated with control technique(s)</td>
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</tbody>
</table>
Table 2-10. Short-term risk management approaches for MC-producing cyanobacteria and MCs

<table>
<thead>
<tr>
<th>Type</th>
<th>MC-producing cyanobacteria (in-lake)</th>
<th>MCs (in-plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical</strong></td>
<td>- Aeration</td>
<td>- Coagulation/Flocculation</td>
</tr>
<tr>
<td></td>
<td>- Coagulation and settling</td>
<td>- Rapid sand filtration</td>
</tr>
<tr>
<td></td>
<td>- Ultrasonication</td>
<td>- Sorption (GAC &amp; PAC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- UV Photolysis</td>
</tr>
<tr>
<td><strong>Chemical</strong></td>
<td>Algaecides:</td>
<td>Oxidants:</td>
</tr>
<tr>
<td></td>
<td>- Copper-based formulations</td>
<td>- Chlorine</td>
</tr>
<tr>
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<td>- Hydrogen peroxide-based formulations</td>
<td>- Potassium Permanganate</td>
</tr>
<tr>
<td></td>
<td>- Endothall formulation</td>
<td>- H₂O₂</td>
</tr>
<tr>
<td></td>
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<td>Advanced oxidation:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ozone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- TiO₂ Photocatalysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- UV + H₂O₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ozone + H₂O₂</td>
</tr>
<tr>
<td><strong>Biological</strong></td>
<td>- Alteration of food web structure</td>
<td>- Slow sand filtration</td>
</tr>
<tr>
<td></td>
<td>- Barley straw</td>
<td>(biodegradation)</td>
</tr>
</tbody>
</table>
### Table 2-11. Examples of integrated management plans in adaptive water resource management

<table>
<thead>
<tr>
<th>Problem</th>
<th>Hypothetical Example 1</th>
<th>Hypothetical Example 2</th>
<th>Hypothetical Example 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water resource use at risk</strong></td>
<td>Drinking water</td>
<td>Recreation/Tourism</td>
<td>Agriculture</td>
</tr>
<tr>
<td><strong>Management goal(s)</strong></td>
<td>Overall goal: total MC concentrations in treated drinking water not to exceed 1 µg/L.</td>
<td>Overall goal: No human exposure to MC-producing cyanobacteria during swimming and no closure of water resource to public</td>
<td>Overall goal: no ingestion of surficial cyanobacterial scum or water containing MCs by cattle</td>
</tr>
<tr>
<td></td>
<td>Immediate goal: manage MC-producing cyanobacteria in the lake to decrease stress on in-plant processes and increase probability of success.</td>
<td>Immediate goal: mitigate MC-producing cyanobacteria from recreational area</td>
<td>Immediate goal: provide alternate drinking water for cattle</td>
</tr>
<tr>
<td><strong>Risk management plan</strong></td>
<td>In-lake: arrange in-lake algaecide application of SCP to mitigate densities of MC-producing cyanobacteria.</td>
<td>In-lake: for immediate mitigation of cyanobacterial densities, apply copper-based algaecide to the cyanobacteria.</td>
<td>In-pond: for immediate mitigation of cyanobacterial densities, apply hydrogen peroxide-based algaecide to the cyanobacteria.</td>
</tr>
<tr>
<td></td>
<td>In-plant: use combination of flocculation, clarification, and filtration to remove cellular MCs. Use PAC or GAC to sorb aqueous MCs. Confirm [total MCs] between treatment steps and in finished water twice daily.</td>
<td>For control throughout the duration of the recreation season, continue to measure cell densities and total MC concentrations in the reservoir and be prepared to trigger management when densities and total MC concentrations exceed the recreation guideline value or standard. If the reservoir is relatively deep (e.g. &gt; 5 m), aeration may be useful to prevent or minimize further colonization. Physical approaches can be evaluated on the basis of site-specific characteristics (depth, volume, surface area) and available budget for management. If pond is &gt; 5 m deep, aeration could be effective. If pond is shallow, periodic algaecide applications may be necessary. Approaches can be evaluated on the basis of site-specific characteristics (depth, volume, surface area) and available budget for management.</td>
<td>For sustained control: evaluate feasibility of physical risk management approaches for MC-producing cyanobacteria. If pond is &gt; 5 m deep, aeration could be effective. If pond is shallow, periodic algaecide applications may be necessary. Approaches can be evaluated on the basis of site-specific characteristics (depth, volume, surface area) and available budget for management.</td>
</tr>
<tr>
<td></td>
<td>For continued control after triage stage: monitor presence and density of cyanobacteria in lake, monitor [MC] in source water, raw water, and treated water. Be prepared for re-development of bloom. Adapt in-plant processes to be robust and effective for MC removal within budget.</td>
<td>For control throughout the duration of the recreation season, continue to measure cell densities and total MC concentrations in the reservoir and be prepared to trigger management when densities and total MC concentrations exceed the recreation guideline value or standard. If the reservoir is relatively deep (e.g. &gt; 5 m), aeration may be useful to prevent or minimize further colonization. Physical approaches can be evaluated on the basis of site-specific characteristics (depth, volume, surface area) and available budget for management.</td>
<td>For sustained control: evaluate feasibility of physical risk management approaches for MC-producing cyanobacteria. If pond is &gt; 5 m deep, aeration could be effective. If pond is shallow, periodic algaecide applications may be necessary. Approaches can be evaluated on the basis of site-specific characteristics (depth, volume, surface area) and available budget for management.</td>
</tr>
</tbody>
</table>


Characterize microcystin (MC) exposures
Source, chemical structures, environmental fate properties, mechanisms of action, spatial and temporal distributions, cellular and aqueous forms

Characterize effects
Potential human exposure routes, complete exposure pathways, ecological toxicity data, species sensitivity distribution

Risk of action vs. no action
Compare potential risk and financial outcomes for decisions of no action, exposure avoidance, and control

Evaluate risk management approaches
For MC-producing cyanobacteria vs. MCs; long term vs. short term; physical, chemical, and biological

Adaptive water resource management
Problem definition, state management goals, develop adaptable plan to achieve goals; integrate multiple risk management approaches

Figure 2-1. Conceptual model for objectives in this paper
**Figure 2-2.** Conceptual model of potential human exposure routes for microcystins.
Figure 2-3. Species sensitivity distribution assembled from ecotoxicological data in peer-reviewed literature
CHAPTER THREE

CELL DENSITY DEPENDENCE OF Microcystis aeruginosa RESPONSES TO COPPER ALGAECIDE CONCENTRATIONS: IMPLICATIONS FOR MICROCYSTIN-LR RELEASE

Abstract

Along with mechanistic models, predictions of exposure-response relationships for copper are often derived from laboratory toxicity experiments with standardized experimental exposures and conditions. For predictions of copper toxicity to algae, cell density is a critical factor often overlooked. For pulse exposures of copper-based algaecides in aquatic systems, cell density can significantly influence copper sorbed by the algal population, and consequent responses. A cyanobacterium, Microcystis aeruginosa, was exposed to a copper-based algaecide over a range of cell densities to model the density-dependence of exposures, and effects on microcystin-LR (MC-LR) release. Copper exposure concentrations were arrayed to result in a gradient of MC-LR release, and masses of copper sorbed to algal populations were measured following exposures. While copper exposure concentrations eliciting comparable MC-LR release ranged an order of magnitude (24-h EC50s 0.03-0.3 mg Cu/L) among cell densities of \(10^6\) through \(10^7\) cells/mL, copper doses (mg Cu/mg algae) were similar (24-h EC50s 0.005-0.006 mg Cu/mg algae). Comparisons of MC-LR release as a function of copper exposure concentrations and doses provided a metric of the density dependence of algal responses in the context of copper-based algaecide applications. Combined with estimates of other site-specific factors (e.g. water characteristics) and fate processes (e.g.
dilution and dispersion, sorption to organic matter and sediments), measuring exposure-
response relationships for specific cell densities can refine predictions for in situ
exposures and algal responses. These measurements can in turn decrease the likelihood
of amending unnecessary copper concentrations to aquatic systems, and minimize risks
for non-target aquatic organisms.
Introduction

Along with mechanistic models (e.g. Biotic Ligand Model), predictions of copper exposures and organism responses are often derived from laboratory toxicity experiments, which aim to standardize exposures (i.e. concentration, duration, frequency, and form) and incubation conditions (i.e. light intensity, temperature, exposure medium), providing the opportunity for comparison of inter- and intralaboratory toxicity data. For predictions of copper toxicity to algae, cell density is an exposure modifying factor often overlooked. Previous studies have shown that apparent toxicity to algae from exposures of certain divalent metals (e.g. Cd, Cu, Zn) decreased as initial cell density increased, likely due to the relative decrease in mass of metal sorbed per mass of algae (Morendo-Garrido et al., 2000; Franklin et al., 2002; De Schamphelaere et al., 2005). To date, research on density dependence of copper exposures has been focused on refining toxicity models (i.e. incorporating cell density) for eukaryotic algal species that serve as primary producers in aquatic systems, in the context of predicting adverse effects that could manifest in community structural changes (i.e. growth inhibition rates; Morendo-Garrido et al., 2000; Franklin et al., 2002; De Schamphelaere et al., 2005). These data are needed for copper-based algaecides for chemical mitigation of harmful algal blooms (HABs) and associated risks (e.g. algal toxins).

As an algaecide, copper is intentionally introduced into aquatic systems in pulse exposures, with the goal of eliciting some targeted level of adverse effects to harmful algae. In order to make accurate predictions of exposures and responses, site-specific factors and processes that could alter exposures should be considered. Intuitively, algal
cells are not solely the intended receptors for these copper exposures, but are also intrinsic exposure modifying factors. The mass of copper sorbed to the algal population is likely driving responses, as opposed to the amended copper exposure concentration alone (De Schamphelaere et al., 2005). As cell density (i.e. algal mass) increases, the mass of copper necessary to elicit a comparable response should increase proportionally, which is the assumption driving this density-dependent exposure hypothesis. In addition to increasing the likelihood of effectively mitigating HABs and preventing unnecessary or excessive copper exposures in aquatic systems, it is anticipated that proactive measurements, or estimates, of initial cell density could also help refine predictions of algal responses like endotoxin release (e.g. microcystins).

Produced by several cyanobacteria genera, microcystins (MCs) are hepatotoxins (Carmichael, 1997; Zurawell et al., 2005) and tumor promoters (MacKintosh et al., 1990; Falconer, 1991) that are toxic to humans (Azevedo et al., 2002), mammals (Fawell et al., 1999; Stewart et al., 2008), aquatic organisms (Zimba et al., 2001), and agricultural crops (Chen et al., 2012; Corbel et al., 2014). Previous studies have contributed to a widely accepted model that assumes MCs completely release from algal cells upon death (Jones and Orr, 1994; WHO, 2011), resulting in restricted uses of copper-based algaecides for cyanobacteria mitigation. Often, studies used to support this leaky cell hypothesis have used unspecified or illegal concentrations of copper algaecides. Recent data to the contrary have shown a relationship between copper exposure concentration and extent of MC release upon cell death (Iwinski et al., 2016). However, among different in situ HAB events, the amended exposure concentration is unlikely to correlate with MC release,
since algal density can vary orders of magnitude from site to site. In this study, modeling changes in MC release following copper algaecide exposures for a range of cell densities provided a metric of the relative change in population-level responses as a function of initial algal mass (i.e. ligand availability). Further, sorbed masses of copper eliciting comparable MC release were compared among densities as an additional line of evidence for density dependence.

In this study, it was necessary to distinguish between the concentration of copper amended to water and the consequent mass of copper in and on algae. A number of definitions are widely accepted and used for the terms exposure and dose (Suter II, 1993; Spacie et al, 1995; Klaassen, 2013). For purposes of this study, exposure was operationally defined as the concentration, duration, frequency, and form of amended copper, while dose was operationally defined as the mass of copper sorbed (adsorbed + absorbed) by a given mass of algae. Laboratory-scale toxicity experiments were conducted with a unicellular cyanobacterium, Microcystis aeruginosa, for a range of cell densities with a strain that consistently produced microcystin-LR (MC-LR). This strain was used so that MC-LR release could be measured reliably, and data could be compared for this well-studied congener (Zurawell et al., 2005; USEPA, 2015). Measurements of chlorophyll-a concentrations provided an additional line of evidence for algal responses to copper exposures. In combination with laboratory physical models, statistical models were used to compare median effect concentrations (EC50s) for MC-LR release in terms of copper exposure concentration and dose, to further elucidate the density-dependence of exposures in this study.
The objectives of this study were to: 1) measure relationships among copper (as copper ethanolamine) exposures and doses for a range of cell densities of *M. aeruginosa* (1x10^6 through 1x10^7 cells/mL), 2) measure responses of *M. aeruginosa* to copper exposures in terms of percent MC-LR release and chlorophyll-a concentrations for each cell density, 3) measure relationships among copper dose and MC-LR release for each cell density, and 4) compare EC50s for MC-LR release in terms of copper exposures and doses among experimental cell densities.
Materials and Methods

Algal culture

Unicellular *Microcystis aeruginosa* (Canadian Phycological Culture Center, Waterloo, ON; CPCC 300) was cultured in ~10 L of BG-11 medium (Grobelaar, 2004) to targeted cell densities of 1x10^6, 5x10^6, and 1x10^7 cells/mL. Water characteristics of the medium were approximately pH = 8.40, dissolved oxygen = 10.2 mg/L, alkalinity = 10 mg/L, hardness = 30 mg/L, and conductivity = 2200 µS/cm. Temperature was maintained at 23±2°C and light was provided at an intensity of approximately 2000-3000 LUX using cool-white fluorescent bulbs (Residential Ecolux 40W, GE) with an 16-h:8-h light:dark cycle. These culture conditions were the same for experimental treatments.

Algal toxicity experimental procedures

Three toxicity experiments were conducted with *M. aeruginosa* at initial cell densities of 1x10^6, 5x10^6, and 1x10^7 cells/mL in BG-11 medium. Copper exposures were prepared from a 1000 mg Cu/L stock solution of a chelated copper algaecide (copper ethanolamine as Cutrine-Plus®; Table 3-1). Ranges of copper exposure concentrations sufficient to measure exposure-response relationships (i.e. partial through maximum measurable response) for each initial cell density were determined with preliminary experiments. Six exposure concentrations were prepared in triplicate (n=3) in 400 mL of BG-11 medium in 500 mL acid-washed borosilicate Erlenmeyer flasks. Untreated controls were algae in BG-11 medium without copper additions. Copper exposures were
measured at test initiation as acid soluble copper using Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP-OES; APHA, 2012).

Measurements of copper doses

Copper dose measurements (as sum of adsorbed and absorbed copper) were modified from methods of Bossuyt and Janssen (2004) and Franklin et al. (2000), and were measured in treatments after 8, 12, and 24-h of exposure to measure maximum copper dose elicited from exposures (sampling frequency determined based on preliminary experiments). Copper doses (existing adsorbed and absorbed copper from growth medium) were measured in untreated controls at experiment initiations. Modification from cited methods included use of 0.45 µm nitrocellulose filter to retain algal cells (rather than centrifugation) between steps for dissolved, adsorbed, and absorbed copper; all other methods were the same as in Bossuyt and Janssen (2004) and Franklin et al. (2000). To measure masses of algal cells, 0.45 µm nitrocellulose filters were first oven-dried at 100˚C for 12-h. Dried filters were cooled to room temperature in a desiccator and weighed prior to filtering known volumes of each cell density. Filters containing algal cells were dried again (same temperature and duration), and weighed. Masses of algae were calculated based on the difference in mass of filters before and after addition of algae on filters, and corrected for the number of cells enumerated in the filtered sample. Copper doses were calculated as the sum mass of adsorbed and absorbed copper per mg algae, although dose has frequently been reported as mass Cu per algal cell. The advantage of reporting copper doses on a cell-by-cell basis is that data can be translated to other cell densities, but the disadvantage is that this method represents a
homogenization of data that may inaccurately predict algal responses on an individual level, since copper in and on a cell is estimated, but not actually measured. Reporting mass of copper per mass of algae could be a more accurate measurement since the mass of algae in the denominator of this ratio (1 mg) can be achieved in the sample taken for copper measurements. Further, this method represents population-level doses to correspond with population-level responses (e.g. MC-LR release), which is a more environmentally relevant relationship in the context of algaeicide applications.

*Algal responses to copper exposures*

Algal responses in terms of total and aqueous MC-LR concentrations were measured synoptically with copper doses at 8, 12, and 24-h. Sampling frequency was determined based on preliminary experiments and previous data for this algal strain exposed to this copper compound (Iwinski et al., 2016). Percent release (proportion of aqueous from total MC-LR concentration) was used as a metric for comparing responses among cell densities. Chlorophyll-α concentrations were measured at 96-h (24-h not sufficient time to discern changes) as an additional line of evidence for algal responses to copper exposures using acetone extraction and spectrofluorometric analysis (APHA, 2012) with a Spectra Max M2® multi-detection microplate reader (Molecular Devices, Sunnyvale, CA).

*Microcystin-LR extraction and quantification*

For aqueous microcystin, 10 mL samples were filtered through a 0.45 µm nitrocellulose filter. Filtrate was concentrated by evaporation using a hot water bath
(~100°C) with constant gentle (i.e. no bubbles generated in sample) air flow directed at head space above samples for approximately 12-h. Once dry, concentrate was re-suspended in 1 mL of 80:20 NANOpure® water: high performance liquid chromatography (HPLC) grade acetonitrile, vortexed to mix, filtered through 0.45 µm nitrocellulose filter, and transferred to 2 mL HPLC vials. Total MC-LR was extracted by first sonicating 10 mL samples to induce cell lysis. Sonication was performed using a sonication probe (Fisher Scientific 550 Sonic Dismembrator®) inserted into each sample for 10 seconds at approximately 180 Watts. Samples were then evaporated for 12-h. Once dry, 1 mL of NANOpure® water was added, samples were vortexed, and frozen at -80°C for 12-h. Samples were then thawed, evaporated to dryness, and re-suspended in 1 mL of 80:20 NANOpure® water: HPLC grade acetonitrile. This solution was then filtered through a 0.45 µm nitrocellulose paper and transferred to 2 mL HPLC vials for analysis. MC-LR concentrations were analyzed using HPLC and standards, instrumentation, and settings in Iwinski et al. (2016).

Statistical Analyses

Linear regression (JMP v.12; SAS Institute, Cary, NC) was used to evaluate relationships among copper exposures and doses for each density, as this was anticipated to be a proportional relationship for each cell density. R² values for goodness of fit, as well as significance tests for non-zero slopes were calculated as explanatory parameters for strength of relationships. Non-linear regression with a sigmoidal fit function was used to analyze responses, in terms of 24-h EC50s for MC-LR release, to both copper exposures and doses. One-way analysis of variance (ANOVA) and linear contrasts were
used to compare chlorophyll-\textit{a} concentrations among untreated controls and copper
treatments. EC50s in terms of copper exposures and doses were compared among cell
densities by comparing 95\% confidence intervals. Graphs were generated using Graph
Pad Prism 7 (GraphPad Software, Inc.; La Jolla, CA).
Results and Discussion

Relationships among copper exposures and doses

Measured copper exposure concentrations were within ± 20% of nominal exposures (data in supplementary material), with the exception of the low density experiment, since relatively low targeted exposure concentrations were used and BG-11 medium has approximately 15 µg Cu/L. Nominal concentrations are reported throughout this paper. Maximum measured copper doses occurred at 12-h, therefore, 12-h copper doses were used for analysis of relationships throughout this study. For the low cell density (1x10^6 cells/mL), copper exposure concentrations ranged from 0.02 to 0.2 mg/L, with associated copper doses ranging from 0.004 to 0.009 mg Cu/mg algae (Figure 3-1A). For the medium cell density (5x10^6 cells/mL), copper exposures ranged from 0.03 to 1 mg/L, with consequent doses of 0.005 to 0.017 mg Cu/mg algae (Figure 3-1B). Finally, for the highest density evaluated (1x10^7 cells/mL), for copper exposures of 0.05 through 2 mg/L, copper doses ranged from 0.0018 to 0.018 mg Cu/mg algae (Figure 3-1C). Copper doses in untreated controls (adsorbed and absorbed copper without algaecide exposures) ranged from 0.0001-0.0009 mg Cu/mg algae. As anticipated, there were measurable positive linear relationships between copper exposure concentration (mg/L) and copper dose (mg Cu/mg algae; Figure 3-1), for all cell densities. Copper exposure concentrations necessary to elicit similar doses among cell densities of 1x10^6 through 1x10^7 cells/mL ranged an order of magnitude.

Resolution to detect changes in dose with exposure increased with increasing cell density, as R^2 values for goodness of fit tests for linear regressions were 0.89, 0.91, and
0.98 for low, medium, and high densities, respectively. Similarly, p values for linear regressions (significance of difference from slope of zero) were 0.0014, 0.0007, and <0.0001 (α = 0.05) for low, medium, and high densities, respectively. These data show that the reliability of measuring adsorbed and absorbed masses of copper may decline at relatively lower cell densities (i.e. < 10⁶ cells/mL).

Responses of M. aeruginosa to copper exposures

Copper exposure concentrations in all toxicity experiments were intentionally arrayed such that majority of the exposures elicited maximum measured algal responses in terms of chlorophyll-a concentrations (i.e. no further decrease in chlorophyll-a concentration with increase in copper concentration). The purpose for this experimental design was to allow for differences in MC-LR release to be discerned among exposures, and ultimately copper doses. Percentages of aqueous MC-LR release from total MC-LR concentrations were used to compare algal responses among cell densities, since total MC-LR can range with cell density (MC-LR concentration data in supplementary material). Maximum measured MC-LR release occurred at 24-h in toxicity experiments, and was therefore used for response analyses. Among each experimental cell density, copper exposure concentration was positively related to MC-LR release (Figure 3-2), particularly within copper exposure concentrations that elicited maximum responses in terms of chlorophyll-a concentrations.

For the low cell density, all copper exposure concentrations from 0.02 to 0.2 mg Cu/L resulted in significant decreases in chlorophyll-a concentrations from untreated controls (p < 0.0001; α =0.05; Figure 3-2A), and chlorophyll-a concentrations among all
exposures were similar (p=0.4926-1.000; α =0.05). Among exposures of 0.02 to 0.2 mg Cu/L, percent MC-LR release ranged from 24% (7 of 28 µg/L total) to 78% (21 of 27 µg/L total), with a 24-h EC50 of 0.03 mg Cu/L. Regarding the medium cell density, copper exposures from 0.1 through 1 mg Cu/L elicited similar responses in terms of 96-h chlorophyll-α concentrations (p=0.6596-0.9998; α =0.05), which were all significantly different from untreated controls (p<0.0001; Figure 3-2B). Among these copper exposures, MC-LR percent release ranged from 44% (146 of 220 µg/L total) to 81% (185 of 227 µg/L total), with a 24-h EC50 of 0.05 mg Cu/L. For the high cell density, copper exposures from 0.1 through 2 mg Cu/L resulted in similar decreases in chlorophyll-α concentrations (p=0.131-1.000 for comparisons among these exposures; α =0.05; Figure 3-2C), with MC-LR release ranging from < 1% (1 of 146 µg/L total) to 92% (123 of 134 µg/L total), with a 24-h EC50 of 0.3 mg Cu/L. It should be noted that 2 mg Cu/L is greater than the legal application (i.e. environmentally relevant) concentration for copper-based algaecides, but in this study was necessary to discern differences in responses up to the cell density of 1x10^7 cells/mL.

Since maximum responses in terms of chlorophyll-α concentration were observed at 0.1 mg Cu/L for both the medium and high cell densities, and MC-LR release was relatively high at this concentration in the medium density, it is likely that the medium density would have responded at an exposure concentration between 0.06 and 1 mg Cu/L that was not evaluated in this study. Lower total MC-LR concentrations in the high cell density than in the medium cell density could have been an artifact of batch cultures, in which nutrients were not continuously added as cell density increased to near carrying
capacity (i.e. nutrients necessary for production of secondary metabolites; Vezie et al. 2002).

These results were similar to findings of Iwinski et al. (2016), where within copper exposures resulting in maximum responses of algae in terms of chlorophyll-a concentrations, gradients of MC-LR release were observed. Among low, medium, and high cell densities evaluated in this study, 24-h EC50s for MC-LR release ranged an order of magnitude in terms of copper exposure concentrations, further demonstrating the role cell density (i.e. mass) plays in these exposure-response relationships. Ultimately, measurements of responses of *M. aeruginosa* to copper exposures served as a confirmation step that this relationship was measurable in this study, supporting further hypotheses of copper dose-microcystin release relationships.

*Relationships between copper dose and MC-LR release and comparisons of EC50s in terms of exposure and dose*

As anticipated, there were measurable sigmoidal relationships among 12-h copper doses and 24-h MC-LR release in this study (Figure 3-3). Calculated 24-h EC50s (in terms of copper dose) for MC-LR release were 0.005, 0.006, and 0.005 mg Cu/mg algae for low, medium, and high cell densities, respectively (Table 3-2). For measured copper doses, both adsorbed and absorbed copper masses increased proportionally with exposure concentrations. Since adsorbed (i.e. surficial) copper should drive uptake rates and extents of copper, both adsorbed and absorbed are appropriate measures of dose and should correlate with algal responses.
For comparisons of EC50s in terms of exposure and dose among cell densities, 95% confidence intervals (CI) were evaluated for overlap, where if CIs overlapped, EC50s were considered similar and if CIs did not overlap, EC50s were considered statistically different. In terms of exposure concentrations, EC50s ranged an order of magnitude (0.03-0.3 mg Cu/L). EC50s for medium and high densities were significantly different (Table 3-2), however, due to a relatively large CI for the low density, the low density did not differ from medium and high densities. Large confidence intervals for the low cell density EC50 (as exposure) could be due to less resolution for MC-LR release among exposures. For EC50s in terms of copper dose, all were similar with overlapping CIs (Table 3-2). Similarities among EC50s in terms of dose show that the mass of copper sorbed to an algal population was proportional to algal response (in terms of MC-LR release) in this study.

Studies conducted in different contexts have also concluded that cell density of an alga can significantly influence the mass of copper sorbed to an algal population and consequently influence responses. For example, Moreno-Garrido et al. (2002) observed an order of magnitude increase in 72-h EC50s with an order of magnitude increase in density for a marine haptophyte, *Isochrysis*, exposed to copper as copper sulfate pentahydrate in laboratory toxicity experiments. Similarly, Franklin et al. (2000) observed decreases in apparent toxicity to 72-h copper exposures as cell densities of two freshwater green algae, *Selenastrum (Pseudokirchneriella)* and *Chlorella* increased.

Since MCs pose ecological and human health risks in aquatic systems, cellular release of MCs is of particular concern when weighing risks of various mitigation options.
for HABs (WHO, 2011). Aqueous MCs can be problematic in potable water resources for which water treatment facilities cannot remove MCs to acceptable guideline concentrations (i.e. ≤1 µg/L; WHO, 2003; WHO, 2011). If potable water resources containing HABs are managed using algaecides, understanding density-dependence can improve the ability to manage exposures of cyanobacteria while minimizing MC release. Cyanobacteria are often more sensitive to copper-based algaecide exposures than non-target algae and aquatic animals (Calomeni et al., 2014; Geer et al., 2016), demonstrating a selective approach for mitigating risks from HABs in water resources. Furthermore, MCs can be subject to biodegradation following algaecide exposures, with half-lives on the order of days (Iwinski et al., 2017), providing a relatively rapid transformation pathway for decreases in MC concentrations and potential risks.

Comparisons of MC-LR release as a function of copper exposure concentrations and doses provided a valuable metric of the density dependence of algal responses in the context of copper-based algaecide applications. However, it would be insufficient to predict copper doses (and consequent responses) based on amended exposure concentrations of algaecides in aquatic systems. These laboratory experiments were designed to model the effects of algal cell density as an exposure modifying factor, however, there are a number of other site-specific factors (e.g. water characteristics) and fate processes that could alter exposures as well (e.g. dilution and dispersion, sorption to other organic matter and sediments). Whereas the purpose of this study was to highlight the significance of cell density (or mass) as one of these factors for consideration,
estimates of all pertinent processes and factors at each specific site are necessary for accurate predictions of exposures and algal responses.
Conclusions

The purpose of this study was to evaluate the influence of *Microcystis aeruginosa* cell density on copper-based algaecide exposures and consequent responses in terms of MC-LR release. The underlying hypothesis was that algal cell density is an intrinsic exposure modifying factor that can influence the mass of copper sorbed by an algal population following copper algaecide exposures. In this study, copper exposure concentrations strongly correlated with copper doses for each cell density evaluated. Further, 24-h median effect concentrations (EC50s) for MC-LR release in terms of copper exposure concentration ranged an order of magnitude with an order of magnitude increase in cell density (0.03-0.3 mg Cu/L for 1x10^6 through 1x10^7 cells/mL). However, in terms of copper dose (sum mass of adsorbed and absorbed Cu/mg algae), 24-h EC50s were similar among all cell densities (0.005-0.006 mg Cu/mg algae). These laboratory physical models, combined with statistical models to estimate and compare EC50s, elucidated the significance of density-dependent exposures for copper-based algaecide applications. Combined with estimates of other site-specific factors (e.g. water characteristics) and processes (e.g. dilution and dispersion, sorption to organic matter and sediments), measurements of cell density prior to copper algaecide applications can refine predictions of in situ exposures and algal responses. Further, refining exposure-response predictions can decrease the likelihood of amending excessive or unnecessary copper concentrations to aquatic systems, as well as minimize risks for non-target aquatic organisms.
References


Calomeni, A., Rodgers, J. H., & Kinley, C. M. (2014). Responses of *Planktothrix agardhii* and *Pseudokirchneriella subcapitata* to Copper Sulfate (CuSO4· 5H2O) and a Chelated Copper Compound (Cutrine®-Ultra). *Water, Air, & Soil Pollution, 225*, 2231.


margins of safety associated with their use. *Archives of Environmental Contamination and Toxicology, 43*, 19-27.


Table 3-1. Physical and Chemical Characteristics of Cutrine-Plus® Algaecide and Herbicide

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Copper (Cu)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>% Active Ingredient</td>
<td>9.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maximum label concentration</td>
<td>1 mg/L (as copper)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Formulation</td>
<td>Copper ethanolamine complex&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Appearance</td>
<td>Blue liquid</td>
</tr>
<tr>
<td>Water Solubility</td>
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<tr>
<td>Boiling Point (°C)</td>
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<td>Specific gravity (g cm&lt;sup&gt;-3&lt;/sup&gt;)</td>
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</tr>
<tr>
<td>pH (S.U)</td>
<td>10.3-10.5&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup>Applied Biochemists product label (2017a)

<sup>b</sup>Murray-Gulde et al. 2002

<sup>c</sup>Applied Biochemists MSDS (2017b)
**Table 3-2.** Comparisons of 24-h EC50s for MC-LR release in terms of copper exposures and doses

<table>
<thead>
<tr>
<th>Experimental Cell Density (cells/mL)</th>
<th>Copper exposure 24-h EC50 for MC-LR release (mg Cu/L) and (95% CI)</th>
<th>Copper dose 24-h EC50 for MC-LR release (mg Cu/mg algae) and (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Low) 1x10^6</td>
<td>0.03 (-0.09-0.14)</td>
<td>0.005 (0.0044-0.0054)</td>
</tr>
<tr>
<td>(Medium) 5x10^6</td>
<td>0.05 (0.03-0.07)</td>
<td>0.006 (0.0047-0.0068)</td>
</tr>
<tr>
<td>(High) 1x10^7</td>
<td>0.3 (0.09-0.4)</td>
<td>0.005 (0.0043-0.0053)</td>
</tr>
</tbody>
</table>
Figure 3-1. Relationships among copper exposures and doses for low (A), medium (B), and high (C) cell densities. Error bars indicate ±1 SD.
Figure 3-2. Responses of *M. aeruginosa* to copper exposures in terms of 24-h MC-LR release and 96-h chlorophyll-a concentrations for low (A), medium (B), and high (C) cell densities. Error bars indicate ±1 SD.
Figure 3-3. Relationships among copper doses and MC-LR release for low (A), medium (B), and high (C) cell densities. Error bars indicate ±1 SD.
CHAPTER FOUR
MICROCYSTIN-LR DEGRADATION FOLLOWING COPPER-BASED
ALGAECIDE EXPOSURES

Abstract

When copper-based algaecides are used in aquatic systems to decrease cyanobacteria densities, endotoxin fate is a concern, due to the potential for human health and ecological risks. Pulse exposures of algaecides can result in episodic low dissolved oxygen (DO) concentrations (< 2 mg L⁻¹), due to oxygen consumed via microbial oxidation of algal detritus. Research objectives of this study were to determine the influence of declining DO levels on microcystin-LR (MC-LR) degradation and changes in resident bacterial assemblages. It was hypothesized that cyanobacteria cell densities would positively correlate with rates and extents of DO decline based on the oxygen required for bacteria to degrade cyanobacteria detritus following exposure to copper-based algaecides. In addition, it was hypothesized that total MC-LR concentrations would increase proportionally with increasing cyanobacteria cell densities. Mesocosm experiments were conducted in a pond in Anderson, SC that frequently experiences cyanobacteria blooms. Three densities of a cyanobacteria assemblage were exposed to a copper ethanolamine algaecide. DO and total MC-LR concentrations were measured with time following algaecide exposures to determine rates and extents of declines. As anticipated, DO concentrations had the highest rate of decline in the highest cell density treatment, followed by medium and low cell densities. MC-LR degradation occurred at similar rates (half-lives 1 to 1.9-d) among cell densities. Acinetobacter and Aeromonas
were dominant in treatments following copper exposures. The relationship between cyanobacteria densities and MC-LR half-lives demonstrates the benefits of managing cyanobacteria in early growth stages to minimize MC concentrations.
Introduction

Several cyanobacteria genera are constitutive or facultative microcystin (MC) producers, posing risks to human health and aquatic ecosystems (Watanabe et al., 1992; WHO, 2011). MCs are hepatotoxins with adverse effects for humans (Azevedo et al., 2002), mammals (Fawell et al., 1999; Stewart et al., 2008), aquatic organisms (Zimba et al., 2001), and agricultural crops (Chen et al., 2012; Corbel et al., 2014). Chemical mitigation of cyanobacteria populations (e.g. with copper-based algaecides) is often an efficient and effective method for decreasing cyanobacteria densities and endotoxin concentrations. There have been concerns regarding MC persistence in aquatic systems following copper-based algaecide applications based on two perceptions. First, copper could have biocidal effects on bacterial assemblages that are capable of MC biodegradation, which could manifest in decreased rates of MC degradation relative to assemblages not exposed to copper algaecides (Jones and Orr, 1994). The rationale for this perception is that since copper can elicit toxicity to cyanobacteria, then other bacteria assemblages could be adversely affected. Second, if rapid onset of oxygen demand occurs, microbial assemblages could be altered in terms of density and diversity, which could also decrease MC degradation rates.

Recent laboratory and field studies were conducted to evaluate if copper could have biocidal properties resulting in prolonged MC persistence (Iwinski, 2016a; Iwinski et al., 2017). In both studies, copper algaecide exposures decreased rates of MC degradation only when copper concentrations were well in excess of legal application concentrations in the US (~ 5 mg Cu L$^{-1}$, where the maximum allowable concentration is
1 mg Cu L\(^{-1}\); Iwinski, 2016a; Iwinski et al. 2017). In comparison, copper algaecide exposures of 0.1 through 2 mg Cu/L resulted in similar degradation rates as untreated controls (degradation of MCs in the absence of copper exposures; Iwinski et al., 2016a; Iwinski et al., 2017). These data provided evidence that MC degradation is unlikely to be impacted by legal copper algaecide concentrations. Both studies were conducted with DO concentrations maintained above 8 mg L\(^{-1}\), in order to evaluate potential adverse effects of copper exposures on microbial assemblages, and consequent indirect effects on MC degradation, while eliminating potential confounding effects of DO concentrations on degradation. To address the second perception, that rapid DO decline after algaecide exposures could impact bacterial assemblages and consequently MC degradation, this study was conducted at the same site with the same copper algaecide used by Iwinski (2016a), without supplying DO.

Until about the last decade, aerobic degradation of MCs was assumed to be more rapid than anaerobic degradation, but more recent peer-reviewed studies reported anaerobic degradation could occur at comparable rates, with half-lives for both processes occurring on the order of days (Cousins et al., 1996; Holst et al., 2003; Edwards et al. 2008; Chen et al., 2010; WHO, 2011; Corbel et al., 2014; Iwinski et al. 2017). These data have shown that both aerobic and anaerobic bacteria are capable of MC degradation under constant DO conditions. Pulse exposures of copper-based algaecides represent a unique situation where portions of normally aerobic systems could become nearly depleted of DO for a period of time (i.e. hours to days), as DO is consumed by microbial degradation of detrital algal cells (Carpenter et al. 1998; Grenz et al., 2000; Wetzel,
As a result of episodic low DO (i.e. < 2 mg L\(^{-1}\)) in aquatic systems, microbial assemblages may be altered in terms of densities and species composition, which could manifest as altered MC degradation rates.

Extent and duration of DO decline following an algaecide exposure are anticipated to correlate with mass of cyanobacteria, since mass of organic matter directly relates to mass of oxygen required for oxidation (Sawyer and McCarty, 1967; Varma and DiGiano, 1968). In addition, total MC concentrations (sum of aqueous and cellular MCs) generally correlate with algal cell densities (Zohary and Pais Madeira, 1990; Chorus and Bartram, 1999). Given these relationships, it is anticipated that the time necessary to reach non-detect concentrations of MCs following a copper algaecide exposure should decrease with decreasing cell density of cyanobacteria present at the time of exposure, due to less cyanobacteria cells (lesser MC concentrations) and presumably less DO demand overall (i.e. lesser impact on bacterial assemblages). In this study, rates and extents of total MC-LR degradation were measured among a range of cyanobacterial densities following copper-based algaecide exposures, with the goal of discerning the relationship between cell density and DO decline, and ultimately cell density and half-lives of MC-LR degradation.

To answer the foregoing questions, field mesocosms provided replicates and captured site-specific characteristics (Graney et al., 1995) which included water and sediments, photoperiod, temperature, and resident bacterial assemblages. Bacterial density, diversity, and relative abundance were measured to confirm that bacterial assemblages were altered following changes in DO concentrations in treatments.
Microcystin-LR (MC-LR) was specifically measured in this study, since peer-reviewed data are available for comparisons of degradation rates for this congener with a range of DO concentrations, with and without copper algaecide exposures. In this context, total MC-LR (sum of aqueous and cellular concentrations) was an appropriate measurement since health advisory guidelines are expressed in terms of total MC-LR (WHO, 2011; USEPA, 2015).

The overall objective of this study was to measure rates and extents of total MC-LR degradation following exposures of a range of cyanobacterial densities to a copper-based algaecide. Specific objectives were to 1) measure responses in terms of cell density of a cyanobacterial assemblage (Microcystis aeruginosa, Planktothrix agardhii, and Anabaena flos-aquae) in mesocosms with a range of initial cell densities (1x10^6, 5x10^6, and 1x10^7 cells mL^{-1}) to 96-h exposures of copper (as copper ethanolamine), 2) measure changes in DO concentrations with time after copper exposures for each cell density, 3) measure and compare rates and extents of MC-LR degradation following copper exposures for each cell density, 4) measure and compare density, diversity, and relative abundance in microbial assemblages among experimental treatments, and 5) compare and contrast data from this study to data with degradation under a range of DO concentrations (with and without copper algaecide exposures) from previous studies.
Materials and Methods

Field site description and preliminary experiments

Mesocosm experiments were conducted at TRI Environmental Inc.’s Denver Downs Research Facility in Anderson, SC (34.580, -82.727) in late August of 2016. Ambient air temperatures during the experiment ranged from 16 to 36˚C. The ponds at this facility receive agricultural and livestock runoff from a surrounding 900-acre farm. The pond where treatments were conducted contained a total phosphorus concentration of approximately 0.3 mg L⁻¹ (determined using inductively coupled plasma with atomic emission spectroscopy [ICP-AES]; APHA, 2012) and a total Kjeldahl nitrogen concentration of approximately 30 mg L⁻¹ (APHA, 2012). Other water characteristics included pH of 8.3, conductivity of 238 µS cm⁻¹, alkalinity of 98 mg L⁻¹ as CaCO₃, hardness of 78 mg L⁻¹ as CaCO₃, and temperature range of approximately 26-31 °C between day and night. pH and conductivity were measured using a Orion® Model 250A pH and meter Triode® electrode, and Orion® Model 142 conductivity meter, respectively. Water temperature was measured via the pH probe and meter. Alkalinity and hardness were measured using titration methods at Clemson University (APHA, 2012). The maximum depth of the pond was approximately 1.5m, while the mean depth was 0.3-0.6m.

At the time of the experiment, this pond contained colonial Microcystis aeruginosa, with relatively lesser abundance of Planktothrix agardhii and Dolichospermum sp. (< 10% of assemblage density), and measurable MC-LR concentrations (~5-50 µg L⁻¹ depending on cell density). Viable cyanobacteria cells were
collected by grab samples with clean 18.9L buckets from the surficial scum. Site water from areas not containing cyanobacteria in the same pond were collected by grab sample with clean 18.9L buckets. Cyanobacteria and water were transported to Clemson University immediately after sampling for preliminary analyses.

Laboratory toxicity experiments were conducted to determine effective copper exposure concentrations for a range of cell densities (1x10^6, 5x10^6, and 1x10^7 cells mL^-1) referred to as low, medium, and high densities, respectively, throughout this paper (Figure 4-1). In laboratory toxicity experiments, exposures were prepared in site water in 250 mL beakers and measured as acid soluble copper using ICP-AES (APHA, 2012). Exposure chambers were maintained in a temperature and light controlled incubator, at 23±2 °C, with lighting provided by cool white fluorescent bulbs (Residential Ecolux 40W, GE) on an 16-h:8-h light:dark cycle. Exposures were not stirred or shaken throughout the 96-h experimental duration.

After 96-h, cell densities and total MC concentrations were measured for untreated controls and copper treatments. Cell densities were measured in unpreserved samples (i.e. immediately following 96-h exposures) using a Leitz Wetzlar Dialux 20 Light Microscope (Leitz USA Scopes, Paramount, CA) and an Improved Neubauer hemacytometer at 250x magnification. Minimum copper exposure concentrations resulting in maximum measured responses (i.e. greatest statistical difference from untreated controls in terms of 96-h cell density) were then targeted in field exposures for each cell density.
Field mesocosm exposures

In situ mesocosms used for field exposures were open-ended high-density polypropylene (HDPE) cylinders. Mesocosms were transported to the site and inserted into the littoral portion along the perimeter of the pond following the completion of the laboratory toxicity experiments (Figure 4-1). Cyanobacteria were amended to enclosures in specific densities by collecting dense surface scums of cyanobacteria from the pond and homogenizing cyanobacteria in a large HDPE horse trough located near the mesocosms. Cyanobacterial cells in the concentrated solution were enumerated in the laboratory, and cyanobacteria were added to individual enclosures to achieve targeted cell densities for low, medium, and high treatments in triplicate based on measured water volumes in enclosures. Untreated controls were targeted cyanobacterial densities in water without additions of copper, for the purpose of measuring total MC-LR concentrations in the absence of a copper algaecide exposure. There were triplicate mesocosms for each of the three cell densities (i.e. experimental treatments), and triplicate mesocosms for untreated controls for each density (18 mesocosms total). Volumes of water within each enclosure were calculated using a cylindrical segment equation (Equation 4-1). Heights from the water surface to the sediment surface were measured on top and bottom of the slope.

\[ V = \frac{1}{2} \pi r^2 (h_1 + h_2) \]  (Equation 4-1)

Copper ethanolamine (as Cutrine-Plus®) was gravimetrically measured to achieve nominal exposures for each enclosure based on the measured volume after algae were
added. Copper exposures were amended to mesocosms at sunrise (7:00 AM) and 50 mL samples were collected for copper exposure concentrations as acid soluble copper in experimental treatments (n=3 for each density). Cyanobacterial responses were measured in terms of cell density (as previously described) after 96-h (Figure 4-1). Differences in cell densities among treatments and untreated controls for each cell density were determined using one-way analysis of variance (ANOVA; JMP v.12).

*Dissolved oxygen measurements*

The frequency of DO concentration measurements was empirically derived. DO measurements were made prior to copper exposures and after 2-h, and frequency of sampling was refined based on these initial measurements in order to obtain resolution for rates of DO decline. DO concentrations were measured using a Hach® portable HDQ meter and an IntelliCAL™ LD0101 Standard Probe (Hach® Loveland, CO). Three measurements were made with depth in mesocosms, evenly spaced from top to bottom of the water column to calculate an average DO concentration in each mesocosm. After the day of algaecide exposures, DO measurements were taken once daily for 15-d around mid-afternoon to measure duration of DO decline relative to pre-treatment concentrations (Figure 4-1).

*Microcystin-LR extraction and quantification*

Subsamples (15 mL) of water and cyanobacteria (stirred in mesocosm to evenly distribute cyanobacteria cells in water column prior to taking samples) were collected in polypropylene centrifuge tubes and transported to Clemson University prior to treatment
and every 12-h (after copper exposures) for total MC-LR analysis for a total of 9-d (when concentrations were non-detect [< 2 µg/L]; Figure 4-1). Total MC-LR was extracted by evaporating 15 ml samples for 8-h using a hot water bath (100 °C) and constant air flow directed at the head space of samples. One mL of NANOpure® water was added to evaporated samples and the samples were frozen overnight at -80 °C, thawed, evaporated, and re-suspended in 1 mL of 80:20 NANOpure® water: HPLC grade acetonitrile. Samples were then sonicated using a Branson® 5210 sonic bath for 10-min, filtered through a 0.45 µm nitrocellulose filter, and transferred to high performance liquid chromatography (HPLC) vials for analysis using HPLC according to Iwinski et al. (2016b).

**Bacterial diversity, relative abundance, and density measurements**

Composite aqueous samples (homogenized cyanobacterial cells and water) from triplicate mesocosms were collected for each treatment and untreated control for measurements of relative abundance and diversity of bacteria 72-h after copper exposures (Figure 4-1). Fifty mL of each sample was filtered through a 0.45 µm Millipore® nitrocellulose filter and DNA was extracted from the filter using the MO BIO PowerWater DNA Isolation Kit. Targeted DNA sequencing was used to identify bacteria present in samples via polymerase chain reaction (PCR) amplification of the v3/v4 region of the 16S ribosomal RNA gene (Klindworth et al., 2013). Library preparation and sequencing was performed as per the manufacturer’s instructions for MiSeq v3 paired-end 300 bp sequencing (Illumina). Library preparation included positive and negative controls, with the former consisting of mock communities, and the latter where no DNA
is added to the PCR, and the sample is carried through to sequencing. After sequencing, the forward and reverse reads were merged using PANDAseq (Masella et al., 2012). All sequences were then filtered and reads were considered to be low quality and discarded if they did not meet the following criteria: average quality greater than Q30, longer than 350 bp, and exact match to the forward primer. Additionally, if the read had any base called as N (unknown) it was discarded. The forward and reverse primers were also removed from each sequence. Bioinformatics pipelines consisting of internally developed scripts and selected QIIME scripts (Caporaso et al., 2010; Edgar, 2010) were used to process the reads. Similar sequences were clustered into groups called Operational Taxonomic Units (OTUs) using a 97% identity threshold and the QIIME pick_de_novo_otus.py script. All OTUs with less than 10 representative sequences across all samples were discarded as a quality filtering step to remove OTUs that may have arisen due to sequencing errors. Taxonomic classification of the OTUs was performed using the Greengenes database version 13_8 (DeSantis et al., 2006; McDonald et al., 2012).

Triplicate samples were collected for each treatment and untreated control for density measurements as most probable number (MPN). Bacterial samples were collected from each mesocosm 72-h post copper exposure (Figure 4-1). Fifty mL samples were collected from each mesocosm and filtered through a 0.45 µm Millipore® nitrocellulose filter. The filtrate was discarded and the filter was stored at -80 ºC in a sterile, 15 mL DNAse/RNase-free polypropylene Nalgene® container until analysis.
MPN of heterotrophic organisms (grown with R2A medium; HiMedia Labs, 2011) were quantified. Samples were diluted 1/5000 with a 0.1% peptone solution. This starting dilution was then diluted serially from 1/50,000 to 1/50,000,000,000. Wells were incubated aerobically at 20 °C without light and assessed for visible growth (formation of a bacterial pellet) after 8-d (when growth ceased to increase through the dilutions).

**Statistical analyses**

MC-LR degradation data for each density were modeled for graphical display in this paper using a four parameter (4P) logistic equation (GraphPad Software, Inc.; La Jolla, CA). However, these models would provide little predictive capability for rate coefficients that could be expected under these experimental conditions. Therefore, data were log transformed, and first order rate coefficients and half-life calculations were used as a metric to compare total MC degradation among cell densities in this study, and among this study and others. Rates and half-lives were calculated based on time required to achieve < 2 µg/L MC-LR from initial concentrations, incorporating lag phases if observed so that rate coefficients were not underestimated. Ninety-five percent confidence intervals were compared among calculated first order rate coefficients to evaluate for statistical difference in rate coefficients among cell density treatments. Densities of bacterial assemblages among untreated controls and treatments were compared using one-way analysis of variance (ANOVA; JMP, v.12; SAS Institute, Cary, NC).
**Comparisons with peer-reviewed data**

Half-lives of MC degradation from this study were compared and contrasted with half-lives measured in previous studies from peer-reviewed literature with a range of DO conditions with and without copper algaecide exposures. Information gathered (if available) included congener(s) of MCs, DO concentration, water temperature, initial MC concentration, and half-lives measured.
Results and Discussion

Responses of the algal assemblage to copper exposures

To support the primary objectives of this study, preliminary laboratory experiments were conducted with site water and cyanobacteria to discern effective copper exposure concentrations for each cell density. In this context, effective copper concentrations were the minimum exposure concentrations eliciting maximum statistical differences in terms of decreased cell density from untreated controls. Based on cell density measurements taken 96-h after copper exposures in laboratory experiments, effective copper exposure concentrations were 0.6, 1.2, and 2.0 mg Cu L\(^{-1}\) as copper ethanolamine for low, medium, and high densities, respectively. For the medium cell density, the highest evaluated concentration (1.2 mg Cu L\(^{-1}\)) did not achieve the extent of cell density decrease from untreated controls measured for low and high densities; therefore, an increased concentration was targeted in situ (1.5 mg Cu L\(^{-1}\)). Copper concentrations above the maximum allowable application concentration (1 mg Cu L\(^{-1}\)) were necessary to accomplish specific objectives in this experiment. Based on these laboratory experiments, targeted concentrations for mesocosm experiments were 0.6, 1.5, and 2.0 mg Cu L\(^{-1}\) for low, medium, and high densities, respectively.

In field mesocosm experiments, average measured copper exposure concentrations were 0.8, 2.0, and 2.5 mg L\(^{-1}\) for low, medium, and high cell densities, respectively, representing a 25-33% difference from targeted concentrations. Disparities in nominal and targeted exposure concentrations were likely due to overestimation of water volume in mesocosms after cyanobacteria were added, however, these differences
were insignificant from targeted copper concentrations for achieving specific objectives in this study. Iwinski et al. (2016a) evaluated copper concentrations from 0.4 to 2 mg Cu L\(^{-1}\) in mesocosm-scale experiments and observed similar half-lives for MC-LR degradation among treatments, indicating that if adverse effects to bacterial assemblages occurred, effects were insufficient to alter degradation rates even when exposed to 2 mg Cu L\(^{-1}\).

Average initial cell densities for low, medium, and high treatments were 1.2x10\(^6\), 6.2x10\(^6\), 1.2x10\(^7\) cell mL\(^{-1}\), respectively. After 96-h copper exposures, average cell densities decreased to 1.6x10\(^4\), 1.1x10\(^5\), and 1.1x10\(^5\) cells mL\(^{-1}\) in low, medium, and high treatments, respectively (Figure 4-2). Average initial cell densities for untreated controls for low, medium, and high treatments were 1.4x10\(^6\), 5.4x10\(^6\), and 1.1x10\(^7\) cells mL\(^{-1}\), respectively. 96-h cell densities in untreated controls were similar to initial densities (low control p=0.2986, medium control p=0.8847, high control p=0.1335; \(\alpha=0.05\)).

_Dissolved oxygen measurements with time_

DO concentrations were measured 2-h after copper exposures, by which time average DO in the high cell density treatments had decreased from 7.1 mg L\(^{-1}\) to 3.4 mg L\(^{-1}\) (Figure 4-3A). After 11-h, low density treatments had an average DO concentration of 6 mg L\(^{-1}\) (from 8.2 mg L\(^{-1}\)), while medium density treatments had 2.4 mg DO L\(^{-1}\) (from 8.1 mg L\(^{-1}\)), and high density treatments had 1.2 mg DO L\(^{-1}\) (from 7.1 mg L\(^{-1}\)). DO in untreated controls for the low density increased from 8 to 12.5 mg/L 11-h after treatment, while DO decreased in controls for medium and high treatments to about 3.5 mg/L by 11-h after treatment. DO decline in untreated controls may have been due to physical
obstruction of atmospheric diffusion (from buoyant cyanobacteria layer), as well as oxygen demand from sediments and water below the cyanobacteria layer. However, there was enough of a difference in DO decline between controls and treatments to parse differences from copper exposures. DO measured 24-h following copper exposures was below 1 mg L\(^{-1}\) for all treatments and untreated controls, likely since these measurements were taken before sunrise (i.e. during respiration). The remainder of the DO data from days 2-15 were measured around mid-afternoon. Since the 24-h data were collected at a different time of day than the other data points, 24-h measurements were not included in Figure 4-3B. DO concentrations were measured for 15 days after copper exposures to discern durations of DO decline from initial concentrations. Three days after copper exposures, DO concentrations in low density treatments increased to above 4 mg L\(^{-1}\), while it took 9-d for the medium density treatments, and 15-d for the high density treatments to increase to above 4 mg L\(^{-1}\) (Figure 4-3B). Therefore, low density treatments had the slowest rate of DO decline and the shortest duration of low DO conditions (i.e. < 2 mg L\(^{-1}\)). Increases in DO concentrations in low density treatments were attributed to photosynthetic DO production via eukaryotic algae (Chlorella sp.) and duckweed (Lemna sp.) that rapidly colonized after cyanobacteria densities decreased (these organisms were not apparent when cyanobacteria were dominant). It was further evident that DO in low density treatments was photosynthetically dependent, due to diurnal changes in DO concentration. DO measured mid-day near the water surface was often above 10 mg L\(^{-1}\) in the low density treatments 3-d after copper exposures. In medium and high density treatments, average DO concentrations remained below 2 mg L\(^{-1}\).
for 7 and 11-d, respectively. Further, both medium and high density treatments developed sulfidic appearance (i.e. black particulates) and odor ~7-d after algaecide exposures. It should be noted that these data are conservative in terms of DO decline following copper algaecide exposures, since measurements were taken in bound enclosures (i.e. no mixing and minimized reaeration due to physical blocking by detrital cells). However, replicated mesocosms provided the ability to achieve specific objectives with statistical power, which included measurements of MC-LR degradation for a range of cyanobacterial masses under low DO (i.e. < 2 mg L\(^{-1}\)) conditions with resident bacteria present.

**Degradation of Microcystin-LR among cell densities**

Average total MC-LR concentrations in untreated controls and treatments prior to copper exposures ranged from 6.7-8.7, 23.4–25.8, and 41-47.3 µg L\(^{-1}\) for low, medium, and high densities, respectively. Two days after copper exposures, MC-LR concentrations in low density treatments decreased to < 2 µg L\(^{-1}\) (Figure 4-4), while 6 and 8-d were necessary for MC-LR concentrations to reach ≤ 2 µg L\(^{-1}\) in medium and high density treatments, respectively. There was an initial lag time (e.g. no statistically significant decreases from initial concentrations) of 3 and 5.5-d in medium and high density treatments, respectively, which could be due to initial changes in microbial densities and/or diversities as a function of rapidly changing DO conditions.

Calculated half-lives of MC degradation based on first order kinetics were similar among treatments (1 to 1.9-d; Table 4-1), where slightly longer half-lives of 1.8 and 1.9-d were estimated for medium and high densities, respectively (Table 4-1), were an artifact
of lag phases. It should be noted that with increasing initial cell density, total MC-LR concentrations increased. Therefore, MC-LR exposures (in terms of total MC-LR concentrations), as well as the duration of time necessary to decrease concentrations below the detection limit (< 2 µg L\(^{-1}\)) decreased with decreasing cyanobacterial density. There were no statistical differences between calculated rate coefficients for each cell density, based on the overlap of 95% confidence intervals (Table 4-1).

There were no measurable decreases in MC-LR in untreated controls for 10-d after copper exposures, with the exception of the low density control (from 8 to 2 µg L\(^{-1}\)). Medium density untreated controls contained an average total MC-LR concentration of 23.4 µg L\(^{-1}\) at experiment initiation and 31.5 µg L\(^{-1}\) 10-d later (day of final MC-LR measurements). Untreated controls for the high cell density contained an average total MC-LR concentration of 41 µg L\(^{-1}\) at experiment initiation and 37.8 µg L\(^{-1}\) at 10-d.

Untreated controls in this study demonstrated trends in total MC-LR concentrations from untreated cyanobacteria assemblages, highlighting consequences of no-action decisions for management of toxin-producing cyanobacteria blooms.

**Microbial density, diversity, and relative abundance**

Bacterial densities among all treatments and untreated controls were not statistically different, due to the relatively large variability in MPN measurements (p=0.4259; \(\alpha=0.05\)). Densities of bacteria were similar among untreated controls for low, medium, and high cyanobacteria cell densities, as well as the low density treatment (~ \(10^4\) MPN mL\(^{-1}\); Table 4-1). Interestingly, average bacterial densities increased 1-2 orders of magnitude in medium and high cyanobacteria cell density treatments. This could imply
that there were greater densities of bacteria associated with greater densities of cyanobacteria upon cell senescence, or that a select number of genera were not affected by relatively high copper exposures (i.e. 2-2.5 mg Cu L\(^{-1}\)) as indicated by diversity data and increase in densities after copper exposures (Table 4-1).

Despite bacteria densities increasing in relation to untreated controls for medium and high cell density treatments, diversities drastically decreased (Table 4-1), indicating there may have been fewer genera that were relatively insensitive to copper exposures applied and/or relatively low DO concentrations. Whereas Simpson’s reciprocal diversity indices ranged from 36-42 in medium and high density controls, indices fell to 11 in both medium and high density treatments (Table 4-1). \textit{Acinetobacter} accounted for 7, 22, and 19\% of microbial community compositions in low, medium, and high cyanobacteria density treatments, respectively (Figure 4-5). Following closely was \textit{Aeromonas}, which accounted for approximately 3, 8, and 16\% of low, medium, and high density treatments, respectively (Figure 4-5).

\textit{Acinetobacter} can colonize aerobic, microaerophillic, and anaerobic systems (Lotter and Murphy, 1985; Lotter et al., 1986), due to its ability to use oxygen (Juni, 1978) and nitrogen (Henriksen, 1976; Lotter, 1985) as terminal electron acceptors. Li et al. (2016) observed that a single strain of \textit{Acinetobacter} completely degraded an initial concentration of 5 \(\mu\)g L\(^{-1}\) MC-LR in 12-h, and also possessed some algaeical capabilities, as observed with \textit{Microcystis aeruginosa}. Similarly, Tsao et al. (2007) identified \textit{Acinetobacter} as a dominant genus present in aerobic MC degradation exposures. Lee et al. (2006) identified \textit{Aeromonas} as a dominant bacterium present on a
granular activated charcoal (GAC) filter exposed to cyanobacteria and aqueous MCs. Other genera that constituted at least 2% of one or more treatments included Acetobacteroides, Cloacibacterium, Clostridium, Delftia, Flavobacterium, and Novosphingobium (Figure 4-5). These genera were not present in appreciable quantities in untreated controls, which provided a comparison for genera that colonized and were potentially responsible for MC-LR degradation following cyanobacterial cell death from algaecide exposures. Unique to this study, these data also indicated genera that rapidly grew under sudden shifts in DO concentrations.

**Comparisons with other aerobic and anaerobic degradation studies**

Similar results have been measured for aerobic and anaerobic MC-LR degradation in other peer-reviewed studies (Table 4-2). For aerobic studies, half-lives have ranged from 2 to 14-d, which are typically correlated with lag phases in degradation (i.e. longer lag phases also followed with longer half-lives; Cousins et al. 1996; Holst et al., 2003; Edwards et al., 2008; Chen et al., 2010; Iwinski et al., 2017). Iwinski et al. (2017) measured half-lives for MC-LR aerobic degradation of about 2 to 2.5-d following copper algaecide exposures of ≤ 2 mg Cu L⁻¹, providing a comparison for aerobic degradation studies with and without copper exposures included.

Under anaerobic conditions (< 1% DO, as operationally defined in Chen et al. 2010) in laboratory experiments conducted at 25 °C using lake-collected sediments as inoculum, Chen et al. (2010) measured decreases in MC-LR concentrations from 50 to < 1 µg L⁻¹ in 8-d following initial lag phases of 2 to 3-d. Holst et al. (2003) measured anaerobic (< 0.3% DO) degradation of MC-LR from 100 to 9 µg L⁻¹ in 1-d using lake
water and surficial sediments in laboratory experiments conducted at 21 °C with additions of 5 mM NO$_3^-$ and 3.3 mM glucose. Following measurements of N$_2$O production in exposures, Holst et al. (2003) suggested that anaerobic MC degradation may be coupled with denitrification near the sediment-water interface. Peer-reviewed data for both aerobic and anaerobic degradation of MCs suggest both are relevant and important removal pathways, and absence of one pathway does not necessarily limit the sum degradation rate of MCs in aquatic systems. However, environmental factors that could influence microbial assemblage capacity for degradation, and potential for lag phases should be considered carefully.

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., 2010). Edwards et al. (2008) concluded prior exposure of a water body to toxin-producing cyanobacteria blooms could increase MC degradation rates, as evaluated using 6 water bodies ranging from no prior blooms to consistent seasonal blooms for several years. Since water temperatures in this experiment ranged from ~25 to 30 °C, which is typical for summer seasons in the southeastern US, temperature was not anticipated to be a limiting factor for MC degradation in this study. The pond used in this experiment has experienced cyanobacteria blooms with production of MC-LR for several years, indicating prior exposure of bacterial assemblages to MC-LR. Lag phases in degradation are often correlated with minimal prior exposure of bacterial assemblages to MCs (Edwards et al.)
2008), however in this study, lag phases were expected to be due to rapid shifts in dissolved oxygen concentrations and/or copper algaecide exposures, given the history of repeated cyanobacterial blooms at the study pond.

Despite measured lag phases in this study, MC-LR half-lives were comparable with other studies. MC-LR degradation rates following copper-based algaecide exposures were of interest in this experiment, due to potential for episodic low DO conditions in aquatic systems. Although half-lives for MC-LR degradation were similar among cell densities, there was a positive relationship between cell density and total MC-LR concentrations, indicating that lower cyanobacterial density resulted in less time required to achieve non-detect MC-LR (i.e. < 2 µg L$^{-1}$). Data from this study demonstrated the benefits of taking action to mitigate cyanobacteria blooms in early growth stages, in terms of minimizing in situ oxygen demand and MC exposures.
Conclusions

MCs are a source of human health and ecological risks, and therefore, fate of MCs in aquatic systems is of concern regardless of form (aqueous or cellular). The impetus for this experiment was anticipated rapid decreases in DO in aquatic systems following cyanobacteria cell death from copper algaecide exposures, and potential for effects of shifts in DO on rates of MC-LR degradation. Rates and extents of MC-LR biodegradation for a range of cyanobacteria densities following copper algaecide exposures were measured. As anticipated, cyanobacteria cell densities rapidly decreased within days following exposure to a copper-ethanolamine algaecide, and rates and extents of DO decline correlated with initial cyanobacteria cell densities. MC-LR degradation occurred at similar rates (half-lives 1 to 1.9-d), with longer half-lives in medium and high density treatments likely an artifact of lag phases (3 to 5.5-d). *Acinetobacter* and *Aeromonas* were dominant bacteria genera in treatments following copper exposures, while these genera occupied minimal fractions of bacterial assemblages in untreated controls, indicating these genera may be able to utilize MCs for carbon and energy, especially in low DO (i.e. < 2 mg L\(^{-1}\)) conditions. MC-LR half-lives measured in this study were comparable with measured half-lives in previous studies with a range of DO concentrations. In this experiment, degradation of MC-LR occurred at environmentally relevant rates (i.e. sufficient rate to minimize concerns of persistent risks in water resources) following copper algaecide exposures for mitigation of cyanobacteria blooms. Further, relationships between cyanobacteria densities and MC-LR degradation half-lives
demonstrated the benefits of taking action in early growth stages to minimize cyanobacteria densities and MC concentrations.
References


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for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal, 6*, 610-618.


Table 4-1. First order rate coefficients, half-lives for MC-LR degradation, and bacterial diversity and density data. NA=not applicable for untreated controls.

<table>
<thead>
<tr>
<th>Cell Density (cells mL⁻¹)</th>
<th>Average MC-LR Half-Life [days (± 2 SD)]</th>
<th>Average $k$ (day⁻¹) (95% CI)</th>
<th>Goodness of Fit: $R^2$</th>
<th>Simpson’s Reciprocal Diversity Index</th>
<th>Average Bacteria Density (MPN mL⁻¹) (± 2 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Control 1x10⁶</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>37</td>
<td>5.9x10⁴</td>
</tr>
<tr>
<td>Low Treatment 1x10⁶</td>
<td>1.0 (1.2)</td>
<td>-0.83 (-5.34 to 3.66)</td>
<td>0.8489</td>
<td>37</td>
<td>2.7x10⁴</td>
</tr>
<tr>
<td>Medium Control 5x10⁶</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>42</td>
<td>6.1x10⁴</td>
</tr>
<tr>
<td>Medium Treatment 5x10⁶</td>
<td>1.8 (0.29)</td>
<td>-0.39 (-0.48 to -0.29)</td>
<td>0.8901</td>
<td>11</td>
<td>5.7x10⁵</td>
</tr>
<tr>
<td>High Control 1x10⁷</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>36</td>
<td>2.6x10⁴</td>
</tr>
<tr>
<td>High Treatment 1x10⁷</td>
<td>1.9 (0.14)</td>
<td>-0.36 (-0.50 to -0.21)</td>
<td>0.7853</td>
<td>11</td>
<td>1.2x10⁶</td>
</tr>
</tbody>
</table>
Table 4-2. Comparison of data from this study with peer-reviewed data for aerobic and anaerobic MC degradation

<table>
<thead>
<tr>
<th>MC Congener</th>
<th>Dissolved Oxygen Conditions</th>
<th>Temp (°C)</th>
<th>Initial [MC] (µg L⁻¹)</th>
<th>Half-Life (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR</td>
<td>Low density&lt; 1-2 mg L⁻¹ Med.density&lt; 1 mg L⁻¹ High density&lt; 1 mg L⁻¹</td>
<td>27</td>
<td>Low density 7±2 Med. Density 26±3 High density 47±6</td>
<td>1 (no lag) 1.8 (includes 3-d lag) 1.9 (includes 5.5-d lag)</td>
<td>This study</td>
</tr>
<tr>
<td>LR</td>
<td>Aerobic (stirred continuously)</td>
<td>21±1</td>
<td>10</td>
<td>3 (reservoir water +sediments) 4.5 (reservoir water alone)</td>
<td>Cousins et al. 1996</td>
</tr>
<tr>
<td>LR</td>
<td>Aerobic (shaken at 100rpm continuously)</td>
<td>29</td>
<td>1000</td>
<td>4-14 (including lag phases 0 to 11-d)</td>
<td>Edwards et al. 2008</td>
</tr>
<tr>
<td>LR</td>
<td>≥ 8 mg L⁻¹</td>
<td>21±2</td>
<td>87±5</td>
<td>2 to 2.5-d</td>
<td>Iwinski et al. 2017</td>
</tr>
<tr>
<td>LR</td>
<td>NS</td>
<td>25</td>
<td>210</td>
<td>3.6 (after 7-d lag)</td>
<td>Lam et al. 1995</td>
</tr>
<tr>
<td>LR, YR, RR</td>
<td>Oxic (Microaerophillic (&lt; 2% O₂) Anoxic (&lt; 0.3% O₂)</td>
<td>NS</td>
<td>Oxic: 70 Microaerophillic: 70 Anoxic: 100</td>
<td>Oxic: ~5 Microaerophillic: ~2.8 Anoxic: ~1</td>
<td>Holst et al. 2003</td>
</tr>
<tr>
<td>LR</td>
<td>Anoxic Aerobic</td>
<td>25</td>
<td>5000</td>
<td>Anoxic: 2.7 (after 2-d lag) Aerobic: 3.9 (after 3-d lag)</td>
<td>Chen et al. 2010</td>
</tr>
</tbody>
</table>
Figure 4-1. Timeline of experiments and data collected. MCs = microcystins; DO = dissolved oxygen.
Figure 4-2. Responses of cyanobacteria assemblage (in terms of cell density) in mesocosms measured 96-h after copper exposures; treatments and controls labeled as initial cell densities, where low = 1x10⁶ cells mL⁻¹, medium = 5x10⁶ cells mL⁻¹, high = 1x10⁷ cells mL⁻¹; error bars represent ± 1 SD.
Figure 4-3. Dissolved oxygen concentrations within the first 12 hours (A) and from days 2-15 (B) after copper algaecide exposures for each cell density; low = 1x10^6 cells mL$^{-1}$, medium = 5x10^6 cells mL$^{-1}$, high = 1x10^7 cells mL$^{-1}$; error bars represent ± 1 SD.
Figure 4.4. Changes in total (sum of cellular and aqueous) microcystin-LR concentrations after copper-algaecide exposures for each cell density; low = 1x10^6 cells mL\(^{-1}\), medium = 5x10^6 cells mL\(^{-1}\), high = 1x10^7 cells mL\(^{-1}\); error bars represent standard error of the mean.
Figure 4-5. Relative abundance of dominant (≥5% of at least one treatment) bacteria genera identified in copper exposure treatments in relation to untreated controls for each algal cell density.
CHAPTER FIVE

SOLAR PHOTOCATALYSIS USING FIXED-FILM TiO₂ FOR MICROCYSTINS
FROM COLONIAL Microcystis aeruginosa

Abstract

Microcystins (MCs) are endotoxins produced by cyanobacteria in freshwaters globally. With known potential for human health risks, rapid and effective treatment methods are needed for MCs. Previous studies have shown photocatalysis can achieve rapid half-lives with UV lamps and slurries of TiO₂. In this experiment, rates and extents of solar photocatalysis of MCs were measured using bench-scale reactors with fixed-films of TiO₂ for solutions with a range of cellular: aqueous MC ratios. Since cellular MCs can be removed physically, photocatalysis rates were measured following sand filtration to discern the extent of MC removal post-filtration. UV energy drives photocatalysis using TiO₂, thus, rates of removal were calculated as a function of cumulative UV insolation and time. For water containing < 10% aqueous MC, filtration removed 90% of total [MC], and the subsequent photocatalysis half-life was 0.37 MJ/m² (or 111-min). For water with ~50% aqueous MCs, filtration removed 52% of total MCs, and the average half-life for photocatalysis was 0.38 MJ/m² (or 138-min). For the > 90% aqueous MC treatment, filtration removed 0% MCs, and the photocatalysis half-life for MCs was 0.37 MJ/m² (or 135-min). Previous studies have used clarified waters; however, results from this study are likely representative of scenarios with waters containing confounding water characteristics and use of solar light for UV, as anticipated in developing countries with less advanced water treatment methods. Photocatalysis is a
rapid and effective process for decreasing concentrations of MCs and could be useful for mitigating risks from MC exposures in drinking water.
Introduction

Microcystins (MCs) are a group of over 100 structural variants (USEPA, 2015) of endotoxins produced by cyanobacteria (Carmichael, 1997; WHO, 2011), with well-studied adverse effects for humans (Azevedo et al., 2002), mammals (Fawell et al., 1999; Stewart et al., 2008), aquatic organisms (Zimba et al., 2001), and agricultural crops (Chen et al., 2012; Corbel et al., 2014). MCs are secondary metabolites (Sivonen, 2009) that can be produced intermittently or consistently by several cyanobacteria that grow in critical freshwater resources used for potable water, recreation, agriculture, industry, and fish and wildlife propagation (Paerl and Otten, 2013). Toxin-producing cyanobacteria are becoming more prevalent in surface water resources, especially at latitudes where there is no previous history of blooms (Lopez et al., 2008; Cheung et al., 2013). Humans can be exposed to MCs via ingestion of drinking water (WHO, 2011; USEPA, 2015), and since freshwater resources for drinking water are limited, efficient and effective treatment methods are urgently needed for managing exposures of MCs.

If consistent MC production by cyanobacteria occurs in a bloom, MC concentrations could correlate with cell density. In many situations, if growth is not prevented or controlled cyanobacteria blooms can achieve relatively high cell densities (e.g. $10^6 – 10^8$ cells/mL), and consequently, relatively high MC concentrations. For human health risks, exposures leading to adverse effects can arise from aqueous MCs in potable water, and an inability of drinking water treatment processes to decrease concentrations below health advisory guidelines (i.e. 1 µg/L total MC-LR [WHO, 2011]; 0.3-1.6 µg/L total MC-LR [USEPA, 2015]).
Management of MC exposures in freshwater resources requires a decrease in concentration, which is a function of transfer and transformation processes specific to these compounds. With molar masses ranging from approximately 800-1,100 g/mol, MCs are cyclic heptapeptides that are resistant to chemical hydrolysis at or near neutral pH (USEPA, 2015). Transformation processes that have relatively rapid half-lives include aerobic and anaerobic biodegradation (i.e. days; Cousins et al., 1996; Edwards et al., 2008; Chen et al., 2010; Iwinski et al., 2017; Kinley et al. 2018) and photolysis (i.e. hours-days; Wormer et al., 2010). In aquatic systems with relatively deep water columns or suspended solids, attenuation of light resulting in minimal UV penetration can decrease the likelihood of photolysis occurring at an appreciable rate (Wormer, 2010). Photocatalysis using titanium dioxide (TiO$_2$) has been investigated as a potential process for rapid removal of MCs in water, with measured half-lives on the order of minutes (Shepard et al., 1998; Lawton et al., 2003; Fotiou et al., 2013).

MCs can exist in cellular and aqueous forms in aquatic systems, and total MC concentrations (sum of aqueous and cellular) have been measured up to parts per million levels (Graham et al., 2010). There are, in general, three plausible scenarios that should be evaluated in the context of photocatalysis treatment of MCs. First, the majority of MCs could be in cellular form (i.e. viable cyanobacterial cells), and conventional drinking water processes including coagulation, flocculation, sedimentation, and filtration should remove most of the MCs from the water and minimal aqueous MCs should require further treatment. The second scenario could be part aqueous, part cellular MCs, which is more likely than the prior scenario, since growths in situ are analogous to “continuous
cultures” and at all times, some cells are growing and some are senescing. It is anticipated that filtration should remove a portion of MCs (in cellular form), and a remaining fraction of MCs would be in the aqueous phase requiring removal. Finally, the worst case scenario would be complete release of MCs from cells, and minimal anticipated removal from water treatment processes designed for physical removal of particulates. This scenario is possible when a bloom senesces rapidly due to heavy precipitation (e.g. flooding events) or temperature (e.g. late in growing season). In this study, sand filters were incorporated to achieve removal of suspended solids (i.e. cyanobacteria cells) prior to photocatalysis for the different scenarios presented above. Sequentially measuring total MC concentrations initially, post-filtration, and post-photocatalysis for each one of the described scenarios can provide context for how efficient each step in the process can be for MC removal under different cellular: aqueous MC ratios.

Previous studies conducted to measure photocatalysis of MCs have often used indoor laboratory experimental designs in which confounding factors were minimized (i.e. clear water with high energy UV exposures), offering a “best-case scenario” estimation of half-lives. Design features (e.g. electric UV light or natural sunlight, slurries or fixed-films of catalysts, indoors or outdoors) can be selected based on the specific needs and capabilities of water treatment facilities. However, in the context of developing countries with less access to advanced drinking water treatment methods, low-cost and low-maintenance designs are especially needed. Immobilizing TiO₂ on a fixed-film eliminates the need to amend and recover catalyst offering greater flexibility in
treatment design. In addition, natural sunlight could provide sufficient energy to accomplish photocatalysis without the use of electric UV light. Results for a treatment design with use of sunlight and a fixed-film of TiO$_2$ can be compared with previous published data to discern the extent to which half-lives can differ under these different circumstances. Since photocatalysis is primarily driven by UV energy (250-400 nm), time is not an adequate comparator for rates and half-lives among different studies (Malato et al., 2009). Calculating rates as a function of cumulative UV insolation could make results from this study widely transferable across seasons and latitudes.

The overall objective of this study was to measure rates of solar photocatalysis for mitigation of MCs using bench-scale reactors with fixed-films of TiO$_2$ following sand filtration. Specific objectives were to 1) measure and compare total MC removal for sand filtration for a range of cellular: aqueous MC ratios (< 10% aqueous MC, ~50% aqueous MC, and > 90% aqueous MC), 2) using filtered site waters, measure rates and half-lives of solar photocatalysis as a function of cumulative UV insolation and time in fixed-film TiO$_2$ reactors, and 3) contrast measured half-lives with half-lives measured in previous studies using different design features (i.e. slurries of catalyst, different UV sources).
Materials and Methods

Source of microcystins and water

Cyanobacteria and water were collected from TRI Environmental Inc.’s Denver Downs Research Facility in Anderson, SC (34°34’46.4"N, 82°43’35.8”W) in mid-September of 2017. The ponds at this facility receive both agricultural and livestock run-offs from a surrounding 900-acre farm. For several years, this pond has experienced seasonal algal blooms of colonial Microcystis aeruginosa spanning from about May-October with consistent production of MCs. Cyanobacteria and water were collected from the site and transported to Clemson University for experiments.

Construction of sand filters and photocatalytic reactors

Small-scale sand filters were constructed in 18.9 L buckets to mimic a rapid gravity (no pressure) sand filtration process. Rapid sand filtration was selected for this study over slow sand filtration, since the goal of the study was to measure MC removal via physical filtration of cells, followed by photocatalysis. Slow sand filtration often involves biological degradation during the filtration process, which would have confounded MC concentrations from filtration to photocatalysis. Rapid sand filtration is more often used in modern drinking water treatment facilities for efficiency (MRWA, 2009). For the sake of simplicity in this experiment, filters were designed for variable declining rate of flow (USEPA, 1974; MRWA, 2009), with filters filled to maximum water capacity, and declining flow rates as pores filled with particles (i.e. cyanobacteria cells and other suspended sediments or organic matter in site water). In the bucket filters,
there was a 12 cm layer of gravel on the bottom to support sand, which was covered with a thin layer (< 1 cm) of polyester fill material to minimize sand loss. Above the gravel and polyester fill layers was a 14.5 cm layer of quartz/silica sand ranging from about 0.4-0.6 mm particle size (MRWA, 2009). Filters had polyvinyl chloride (PVC) open-ended elbow joints installed at the bottom of the bucket on the side (adjacent to gravel layer) to drain filtrate. Prior to filtering site water and cyanobacteria for the experiment, each filter was rinsed with a continuous flow of distilled water for approximately 1-h, to saturate and compact sand and rinse any fine particles from filters.

Photocatalytic reactors were constructed in 28x43 cm Sterilite® high-density polyethylene (HDPE) containers. A thin-film of epoxy resin and hardener (105 Epoxy Resin; 206 Hardener; West Marine Products, Inc., Watsonville, CA) was applied as a <1 mm film onto a stainless-steel plate followed by the application of TiO₂ (Aeroxide™ P25; Fisher Scientific, Fairlawn NJ). Stainless-steel plates with fixed-film TiO₂ were cured at ambient air temperature (~20°C) for 1-wk. Following curing, each stainless-steel plate (i.e. reactor) was rinsed 3x with distilled water to remove any loosely bound particles and then placed in a clean HDPE container, so that the catalyst would be in contact with overlying site water. The TiO₂ used had a crystalline composition of 10-20% rutile and 80-90% anatase, was approximately 45 µm or greater, and had a specific surface area of 35-65 m²/g.

**Experiment design and procedure**

Experimental treatments were designed to simulate the three potential scenarios previously described. First, site water and a relatively high density of *M. aeruginosa*
(1x10^7 cells/mL) with a total MC concentration of approximately 25 µg/L (as MC-LR equivalents) were stirred in a clean 56 L plastic bin to evenly distribute cyanobacteria cells in the site water. To create ratios of cellular and aqueous MCs, known volumes of a 100% aqueous MC solution, live cyanobacteria, and site water (no cyanobacteria) were mixed. To create a 100% aqueous MC solution, approximately 15 L of viable cyanobacteria in site water was collected and sonicated using a sonication probe (Fisher Scientific 550 Sonic Dismembrator®) inserted into volumes of 1 L for 15-s at approximately 180 W. Solutions were then frozen at -80°C for 12-h and thawed.

The first treatment (< 10% aqueous MC) was prepared with 6 L of site water, 5.5 L of viable cyanobacteria, and 0.5 L of the sonicated and frozen solution for a total volume of 12 L used to distribute among replicate reactors. The second treatment (~50% aqueous MC) was prepared with 6 L of site water, 3 L of viable cyanobacteria, and 3 L of sonicated and frozen cyanobacteria. The third treatment (> 90% aqueous MC) was prepared with 6 L of site water and 6 L of sonicated and frozen cyanobacteria. Controls for this experiment included a dark control to account for potential sorption to catalyst surfaces, in which reactors were covered with opaque lids and wrapped in black opaque plastic bags to prevent light penetration. “Photolysis only” controls were in HDPE containers without catalysts, to parse rates of removal in the absence of the catalyst. Dark and photolysis only controls were prepared the same way as the ~50% aqueous MC treatment, so that there were sufficient concentrations of MCs to measure changes in concentration if applicable. All treatments and controls were conducted with 3 independent reactors. Triplicate 1 mL samples were collected in acid-washed
borosilicate glass vials from each of the 3 treatments and 2 controls for quantification of initial total (sum of cellular and aqueous) MC concentrations.

After treatment and control mixtures of cyanobacteria and site water were prepared, each was poured through a sand filter bucket (5 sand filters, one for each of 3 treatments and 2 controls). Once filtrate drained from filters into clean 5 gallon buckets, triplicate samples were collected from each treatment and control for “post-filter” total MC concentrations. Buckets were then transported outdoors to initiate the photocatalysis portion of the experiment. Photocatalysis reactors were located on the roof of a building to prevent any confounding shading or interference with samples. Three and a half liters were introduced into each reactor for all treatments and controls. To account for evaporation during the experiment (for accurate calculation of rates), volumes in reactors were measured and re-constituted with fresh site water (containing no measurable MC) twice; once at approximately the middle of the experiment (180-min) and once at the completion of the experiment (340-min), and rates of evaporation were averaged and corrected for MC analytical measurements.

The experiment was initiated at 11:50 AM in mid-September of 2017 with clear, sunny skies and ambient air temperatures of 24-29°C. UV irradiance was measured every minute for the duration of the experiment using an Apogee Instruments SU-100 UV sensor (Logon, UT; spectral range: 250-400 nm) and a HOBO® UX120-006M Analog Data logger (Onset® Computer Corporation, Bourne, MA) to calculate cumulative UV insolation. Water characteristics measured in the site water used for treatments included dissolved oxygen, pH, and conductivity using a YSI® Model 52 dissolved oxygen meter,
Orion® Model 250A pH meter and Triode® electrode, and Orion Model 142 conductivity meter, respectively. Alkalinity and hardness of aqueous samples were measured according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Turbidity of the site water was measured using a turbidimeter (LaMotte 2020e, LaMotte Company, Chestertown MD, 21620), and dissolved organic carbon (DOC) concentration in site water was measured using high-temperature combustion (Standard Method 5310B; APHA, 2012).

MC-LR exposure preparation and quantification

Quantitative measurements of total MCs were conducted according to Ohio EPA’s Standard Operating Procedure (OHEPA, 2015), using an Adda Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well microtiter plate (Abraxis Inc., Warminster, PA 18974; PN 522015). The analytical method detection limit (MDL) for this assay was approximately 0.3 µg/L as total MCs determined using OHEPA’s SOP for determination of MDL (OHEPA, 2015). To measure the sum of cellular and aqueous MCs, cells were lysed using a repeated freeze-thaw sequence (3x at -20°C; OHEPA SOP, 2015). Samples were kept frozen until analysis, and all samples reported in this manuscript were analyzed within 24-h of experiment completion. Immediately prior to analysis using ELISA assays, samples were filtered using a 0.45µm glass fiber filter. Quality assurance and quality control (QA/QC) procedures for each batch of samples analyzed included comparisons of measured absorbance to a standard curve (0-5 ug/L as MC-LR), analysis of laboratory reagent blank (solution used to dilute samples for analysis), analysis of a low calibration range sample (0.4 µg/L MC-LR), and a quality
control standard (secondary source of MC-LR with known concentration of 0.75 µg/L).

All samples, including standards and blanks were analyzed in duplicate wells, and the average absorbance for each was used to calculate concentrations of total MCs (as MC-LR equivalents).

**Percent removal by filters and photocatalysis rate calculations**

Percent removal of total MC concentrations by sand filters (equation 1) was estimated using the following equation:

\[
\text{Removal efficiency (\%)} = \left( \frac{[C_0] - [C]}{[C_0]} \right) \times 100 \tag{1}
\]

Where, measured initial concentrations are designated as \([C_0]\) (µg/L) and \([C]\) (µg/L) is concentration in filtrate. In addition, removal rate coefficients as a function of cumulative UV insolation (MJ/m\(^2\))\(^{-1}\) and time (min\(^{-1}\)) were estimated using first order rate kinetics using the following equation:

\[
\text{Removal rate coefficient (k)} = \frac{-\ln([C]/[C_0])}{\text{UV or t}} \tag{2}
\]

Where, UV (MJ/m\(^2\)) is cumulative UV insolation and \(t\) is time (min). Removal rate coefficients (MJ/m\(^2\))\(^{-1}\) or (min\(^{-1}\)) represent the slope of the line obtained by plotting – \(\ln([C]/[C_0])\) versus cumulative UV or time. Half-lives (in terms of cumulative UV or time) were calculated using first order kinetics, equal to the natural log of 2 (=0.693) divided by the rate coefficient.
Compare and contrast design

Results from this study were compared and contrasted with results from other peer-reviewed studies in terms of photocatalysis half-lives. Since previous studies calculated half-lives in terms of time, those data were reported for context. Design parameters that were included in comparisons were source of UV (type, spectral range, and power if reported), medium for photocatalysis, form of catalyst, initial MC concentration, and half-life.
Results and Discussion

*Water characteristics and extents of total MC removal from sand filtration*

Site water used in this experiment had a pH of 8.04 (S.U), dissolved oxygen concentration of 8.56 mg/L, conductivity of 2202 µS/cm, alkalinity of 100 mg/L as CaCO₃, hardness of 125 mg/L as CaCO₃, turbidity of 19.9 NTUs, and DOC concentration of 9±2 mg/L. Initial total MC concentrations (as MC-LR equivalents) were 9.7 (±1.9), 11.1 (±1.3), and 11.1 (±1.4) µg/L in < 10% aqueous, ~50% aqueous, and > 90% aqueous MC treatments, respectively (Table 5-1). In photolysis only and dark controls, initial total MC concentrations were 10.2 (±2.1) and 11.2 (±2.8) µg/L, respectively (Table 5-1). Following sand-filtration, the < 10% aqueous MC treatment contained 0.95 (±0.09) µg/L, resulting in 90.2% removal of total MC prior to photocatalysis (Table 5-1). For the ~50% aqueous MC treatment, filtrate contained an average total MC concentration of 5.3 (±1.3) µg/L, resulting in 52.3% removal of total MC prior to photocatalysis (Table 5-1). The >90% aqueous MC treatment contained 12.7 (±1.1) µg/L as total MC-LR equivalents following sand filtration (Table 5-1). Although the average MC concentration for this treatment was slightly higher than the average initial total concentration, averages were within the variability of each measurement. Photolysis only controls contained 6.1 (±1.7) µg/L following sand filtration, indicating 40% removal of total MCs (Table 5-1). For dark controls, 6.8 (±0.96) µg/L remained in solution following filtration, indicating 39% removal from total initial concentrations (Table 5-1). Based on complete recovery of aqueous MCs from initial total concentration to the filtered total concentration in the
>90% aqueous MC treatment, no measurable sorption of MCs occurred in sand filters during filtration.

These data illustrated the effectiveness of filtration for removing MCs when the majority of MCs are in cellular form. Since cyanobacteria undergo continuous growth phases in situ, there are likely minimal fluctuations in cellular: aqueous MC ratios throughout a growing season. More likely, there is some continuous aqueous MC concentration as cells grow and senesce. When day lengths shorten and temperatures cool at the end of a growing season, cells are more likely to senesce instantaneously, resulting in rapid release of majority of MCs within a short period of time (hours). Based on this information, filtration would likely be more effective for removal of MCs in the early stages of a bloom.

Photocatalysis rates and extents of removal

Photocatalysis was conducted for a total of 340-min (5.7-h), and in that duration, the cumulative UV insolation measured was 0.924 MJ/m². Cumulative UV insolation at designated sampling times was also calculated to determine rates of removal as a function of cumulative UV (Table 5-2). Calculated rate coefficients and half-lives based on first order kinetics are presented in this paper, although it should be noted calculated rates and half-lives in terms of both cumulative UV insolation and time were more conservative than measured rates and half-lives (Table 5-3). For the < 10% aqueous MC treatment, MC concentrations decreased from 0.95 (±0.09) µg/L to the MDL (0.3 µg/L) at a cumulative UV insolation of 0.588 MJ/m² (180 min; Figure 5-1), yielding an average removal rate coefficient of -1.93 (MJ/m²) · and an average calculated half-life of 0.37
MJ/m² (Tables 5-2 and 5-3). For the ~50% aqueous MC treatment, MC concentrations decreased from 5.3 (±1.3) to 0.8 (±0.09) µg/L at a cumulative UV insolation of 0.924 MJ/m² (340 min; Figure 5-1), resulting in an average rate coefficient of -1.81 (MJ/m²)⁻¹ and an average calculated half-life of 0.38 MJ/m² (Tables 5-2 and 5-3). For the > 90% aqueous MC treatment, initial MC concentrations decreased from 12.7 (±1.1) to 1.8 (±0.6) µg/L at 0.924 MJ/m² (340 min; Figure 5-1), with an average removal rate coefficient of -1.85 (MJ/m²)⁻¹ and an average calculated half-life of 0.37 MJ/m² (Tables 5-2 and 5-3). For the photolysis only control, initial MC concentrations averaged 6.1 (±1.6) µg/L and decreased to 2.8 (±0.4) µg/L at 0.924 MJ/m² (340 min; Figure 5-1), with an average removal rate coefficient of -0.72 (MJ/m²)⁻¹ and an average calculated half-life of 1.02 MJ/m² (Tables 5-2 and 5-3). Photocatalysis rates were approximately 2.3x faster than the rate of photolysis alone. In dark controls, MC concentrations were similar throughout the duration of the experiment, as the initial MC concentration was 6.8 (±0.9) µg/L and the concentration at the end of the experiment was 6.5 (±0.6) µg/L at 340-min (final sampling time in experiment), indicating no measurable sorption to catalyst surfaces in reactors.

Photons in the solar UV spectral range (i.e. 300-390nm) have sufficient energy to generate electron pair holes in TiO₂ (Malato et al., 2009), however, anticipated rates could change as a function of factors that influence the quantity of photons reaching the catalyst surface (e.g. altitude angle of the sun). UV irradiance (measured once every 60-s) ranged from 49-54 W/m² within the first 3-h of the experiment (11:50 AM – 2:50 PM), and then steadily declined until sunset. When UV energy is absorbed by TiO₂, the
number of electrons and holes increase in proportion to the light intensity (Hu, 2010), where light intensity is proportional to the number of photons interacting with the catalyst. However, the concentration of reactant in solution (compound undergoing photocatalysis) also influences photocatalysis rates, since generated radicals can only be effective in close proximity to an oxidizable compound (Malato et al., 2009). If less reactant is available for oxidation in solution, then fewer radicals should be necessary at any given time, which could explain why decreases in MC concentrations appeared to track with cumulative UV insolation despite the decrease in UV irradiance with time.

Comparisons to previously published data

Although time is not an adequate comparator for photocatalysis rates among studies (Malato et al., 2009), half-lives in terms of time are generally reported in peer-reviewed literature, and can be used for baseline comparisons given that data are interpreted with the experimental conditions used. Half-lives for photocatalysis of MCs in terms of time in this study were relatively longer than half-lives reported in previous studies (Table 5-4), which was anticipated given the types and intensities of UV energy used. Feitz et al. (1999) compared rates of photocatalysis for MC-LR extracted from cyanobacteria for a UV lamp versus sunlight with a TiO$_2$ slurry in Milli-Q water (Feitz et al., 1999). Feitz et al. (1999) measured irradiance of 520 W/m$^2$ from photons with wavelengths of 365 nm and an experimental duration of 30-min (in this time, MC-LR concentrations decreased from 85 to 1 µg/L). Assuming this irradiance was uniform throughout the experiment, cumulative UV insolation in this case would have been approximately 0.94 MJ/m$^2$ for the 30-min experiment, similar to the solar insolation that
was measured for the entire experimental duration in this study (0.92 MJ/m² in 340-min). In the solar experiment, a slurry of 1 g/L TiO₂ and approximately 560 µg/L MC-LR (crude extract) were mixed in 20 mL of Milli-Q water in a small beaker and placed outside in direct sunlight (Feitz et al., 1999), and concentrations decreased to approximately 1.5 µg/L in 40-min. Feitz et al. referenced a solar irradiance of 40 W/m² from Matthews and McEvoy (1992), but did not measure actual irradiance in their experiment. Since location and date of the solar experiment were not reported, the likelihood that actual irradiance was similar to the referenced value is unknown.

The majority of studies used controlled wavelength and energy lamps, with some in the higher energy spectra (< 300 nm) than actual wavelengths reaching earth’s surface from sunlight (300-390 nm; Malato et al., 2009). In addition, the majority of studies reviewed used waters without DOC (other than MCs) or turbidity present (i.e. distilled and Milli-Q waters), both of which can 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 TiO₂ exposed to 8-30W 254 nm lamps and measured half-lives of < 5-min. Comparatively, with lake water, the half-life for MC-YR increased to 21.3-min 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-min in distilled water and 6-min in lake water (pH 8; Shepard et al. 2002). Unfortunately, neither DOC concentrations nor turbidity were reported for lake water samples in these studies. Measured water characteristics included a chemical oxygen demand of 34 mg/L, ammonia concentration of 0.02 mg/L, nitrate and nitrite concentrations summed of 0.47 mg/L, and soluble
reactive phosphorus of 0.205 mg/L (Shepard et al., 2002). Shepard et al. (2002) reported a theoretical irradiance of 204.5 W/m² based on lamps used in laboratory studies with both distilled and lake waters, which is equivalent to 0.012 MJ/m²/min, yet since total irradiation time necessary to achieve reported results was not reported, a cumulative UV insolation could not be estimated.

Experiments conducted by Lawton et al. (1999 and 2003) used lamps emitting photons in the UV-A and visible light ranges. Lawton et al. (1999) measured half-lives of approximately 2-min for MC-LR purified from *Microcystis aeruginosa* in Milli-Q water, in solutions of 1% m/v TiO₂ that were continuously stirred at pH 4 and 32.8°C and exposed to 280 W lamps emitting wavelengths from 330-450 nm (Table 5-4). In a subsequent experiment, Lawton et al. (2003) measured a removal rate of 15 µg/mL/min at pH 7 in a laboratory experiment, in which MCs were spiked in Milli-Q water with a slurry of TiO₂ exposed to 480 W bulbs emitting 330-450 nm wavelength photons. Fotiou et al. (2013) conducted an experiment in which 5 mL of Milli-Q water containing 10 mg/L MC-LR and 200 mg/L TiO₂ was aerated in a cylindrical cell for 20-min and then exposed to 4-15W bulbs (UV-A: 365nm; measured irradiance of 717 W/m²) with continuous stirring. MC-LR concentrations decreased to non-detect concentrations within about 12-min for Degussa TiO₂ and in just over 20-min for an immobilized film of TiO₂ using graphene oxide. Using a solar apparatus designed to emit energy from the UV and visible light spectrum at a zenith angle of 48.2° (measured irradiance of 1150 W/m²), MC-LR decreased from 10 mg/L to non-detect in approximately 15-min and 2-h for a slurry of Degussa TiO₂ and an immobilized film of TiO₂ using graphene oxide,
respectively (Fotiou et al., 2013). For context, the measured irradiance in the UV spectrum (250-400nm) in this study ranged from 49-54 W/m² between 11:50AM and 2:50PM, which is roughly a factor of 22 less than that measured from the solar apparatus used by Fotiou et al. (2013).

It is clear that time alone is insufficient for comparisons of rates of photocatalysis among studies. In a review of the kinetics of photocatalysis using semi-conductors, Malato et al. (2009) stated that use of illumination time as the calculation unit for rates could result in misinterpretation of data, since differences in incident UV radiation are not taken into account. UV energy is a necessary parameter for accurate evaluation of results, and broadband UV radiation can be considered an appropriate spectral range for standardization of data among studies (Malato et al., 2009). Since photocatalysis is a promising treatment process for MCs in surface waters globally, these experimental parameters are necessary for accurate comparisons of inter- and intralaboratory data, with the ultimate goal of predicting removal rates under different conditions.

Based on previous studies conducted to measure photocatalysis that used electrically sourced UV light with roughly an order of magnitude greater irradiance than that of solar photons, there is evidence that under “best-case scenario” treatment situations (i.e. waters with minimal turbidity and DOC), MCs can be removed from waters with half-lives of less than 10-min. These scenarios could exist in modern drinking water treatment facilities that can eliminate confounding suspended solids and dissolved organics during initial treatment processes (e.g. sedimentation, coagulation,
flocculation, filtration, and oxidation) and use electric sources of UV for indoor photocatalysis treatment. In this study, treatments were designed to collect photocatalysis data for solar UV and site water with relatively higher turbidity and DOC concentrations than previously evaluated. These scenarios are more likely in developing countries that often use unfiltered and untreated surface waters for drinking waters (Funari and Testai, 2008). Measured half-lives for solar photocatalysis using a fixed-film of TiO$_2$ in terms of time in this study were rapid (on the order of 111 to 138-min) as compared to other transformation processes for MCs, including aerobic and anaerobic degradation (half-lives of days). Combined with filtration (i.e. sand filters), simple fixed-film reactors could be designed to aid with drinking water issues related to MCs in developing countries, which is urgently needed for decreasing potential for human-health risks. Similarly, in developed countries where majority of surface waters are used for drinking water and are simultaneously impacted by HABs, this technology is also needed.
Conclusions

The goal of this study was to measure rates of solar photocatalysis of MCs using a fixed-film of TiO$_2$ preceded by sand filtration of waters with a range of cellular: aqueous MC ratios. A secondary objective was to calculate rates of photocatalysis as a function of cumulative UV insolation, since UV energy ultimately drives this process. The final goal was to compare and contrast data from this study with previously published data for different experimental conditions. For water containing < 10% aqueous (> 90% cellular MC), sand filtration removed approximately 90% of total [MC], and the subsequent photocatalysis half-life averaged 0.37 MJ/m$^2$ (or 111-min). For the treatment consisting of ~50% aqueous MCs, sand filtration removed about 52% of total MCs, and the average half-life for photocatalysis was 0.38 MJ/m$^2$ (or 138-min). Finally, for the > 90% aqueous MC treatment, sand filtration removed 0% MCs, and the half-life for photocatalysis averaged 0.37 MJ/m$^2$ (or 135-min). As compared to previously conducted laboratory studies for this process, measured half-lives in this study in terms of time were relatively longer, which is likely due to decreased irradiance from the sun as compared to electric UV lamps, as well as confounding water characteristics including turbidity and DOC. Previously conducted studies can provide predictions for situations in which waters that can be clarified of suspended solids and organic matter prior to treatment, and electric UV irradiance can be implemented. However, without UV irradiance data, comparisons of photocatalysis rates among studies should be made with caution. Results from this study are likely representative of treatment scenarios with waters containing confounding characteristics for treatment (i.e. turbidity and DOC) and sunlight as the UV source,
which is more likely in developing countries with less advanced (i.e. energy-demanding methods) for drinking water treatment. Photocatalysis is a rapid and effective process for decreasing concentrations of MCs. Early detection and treatment of MCs using photocatalysis could be useful for mitigating risks from MC exposures in drinking water resources.
References


Table 5-1. Average initial total MC and post-filter MC concentrations measured prior to photocatalysis experiment.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10% Aqueous MC</td>
<td>9.7 (1.9)</td>
<td>0.95 (0.09)</td>
<td>90.2</td>
</tr>
<tr>
<td>~ 50% Aqueous MC</td>
<td>11.1 (1.3)</td>
<td>5.3 (1.3)</td>
<td>52.3</td>
</tr>
<tr>
<td>&gt; 90% Aqueous MC</td>
<td>11.1 (1.4)</td>
<td>12.7 (1.1)</td>
<td>0</td>
</tr>
<tr>
<td>Photolysis Only</td>
<td>10.2 (2.1)</td>
<td>6.1 (1.7)</td>
<td>40</td>
</tr>
<tr>
<td>Dark Control</td>
<td>11.2 (2.8)</td>
<td>6.8 (0.9)</td>
<td>39</td>
</tr>
</tbody>
</table>
Table 5-2. Changes in total MC concentrations with time (min) and cumulative UV insolation for solar photocatalysis and controls.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (min)</th>
<th>Cumulative UV Insolation (MJ/m²)</th>
<th>Total MC Concentration [as µg/L MC-LR equivalents] [+SD]</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10% Aqueous MC</td>
<td>0</td>
<td>0</td>
<td>0.95 (0.09)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.094</td>
<td>0.56 (0.04)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.191</td>
<td>0.52 (0.03)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.29</td>
<td>0.38 (0.07)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.417</td>
<td>0.36 (0.04)</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.588</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>~ 50% Aqueous MC</td>
<td>0</td>
<td>0</td>
<td>5.3 (1.3)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.191</td>
<td>3.5 (0.4)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.29</td>
<td>2.2 (0.6)</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.588</td>
<td>1.6 (0.2)</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>0.694</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>0.848</td>
<td>1.1 (0.09)</td>
</tr>
<tr>
<td></td>
<td>340</td>
<td>0.924</td>
<td>0.8 (0.09)</td>
</tr>
<tr>
<td>&gt; 90% Aqueous MC</td>
<td>0</td>
<td>0</td>
<td>12.7 (1.1)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.191</td>
<td>6.2 (0.01)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.29</td>
<td>5.9 (2.2)</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.588</td>
<td>3.3 (0.5)</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>0.694</td>
<td>2.9 (0.3)</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>0.848</td>
<td>2.6 (0.5)</td>
</tr>
<tr>
<td></td>
<td>340</td>
<td>0.924</td>
<td>1.8 (0.6)</td>
</tr>
<tr>
<td>Photolysis Only</td>
<td>0</td>
<td>0</td>
<td>6.1 (1.6)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.191</td>
<td>6 (0.5)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.417</td>
<td>5.3 (0.2)</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.588</td>
<td>4 (0.3)</td>
</tr>
<tr>
<td></td>
<td>340</td>
<td>0.924</td>
<td>3.3 (0.4)</td>
</tr>
<tr>
<td>Dark Control</td>
<td>0</td>
<td>0</td>
<td>6.8 (0.9)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.191</td>
<td>6.8 (0.4)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.417</td>
<td>6.6 (0.3)</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.588</td>
<td>6.4 (0.5)</td>
</tr>
<tr>
<td></td>
<td>340</td>
<td>0.924</td>
<td>6.7 (0.4)</td>
</tr>
</tbody>
</table>
Table 5-3. Calculated rate coefficients and half-lives based on time and cumulative UV insolation using first-order kinetics. NA= not applicable.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average 1st order rate coefficient for photocatalysis (min⁻¹) (range)</th>
<th>Average Half-life (min) (±SD)</th>
<th>Average 1st order rate coefficient for photocatalysis (MJ/m²) (range)</th>
<th>Average Half-life (MJ/m²) (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10% Aqueous MC</td>
<td>-0.006 (-0.0048 to -0.0075)</td>
<td>111 (28.6)</td>
<td>-1.93 (-1.45 to -2.22)</td>
<td>0.37 (0.09)</td>
</tr>
<tr>
<td>~ 50% Aqueous MC</td>
<td>-0.005 (-0.0043 to -0.0058)</td>
<td>138 (21.2)</td>
<td>-1.81 (-1.52 to -2.07)</td>
<td>0.38 (0.06)</td>
</tr>
<tr>
<td>&gt; 90% Aqueous MC</td>
<td>-0.005 (-0.0046 to -0.006)</td>
<td>135 (17.9)</td>
<td>-1.85 (-1.61 to -2.15)</td>
<td>0.37 (0.05)</td>
</tr>
<tr>
<td>Photolysis Only</td>
<td>-0.002 (-0.0014 to -0.0025)</td>
<td>373 (111.3)</td>
<td>-0.719 (-0.5 to -0.89)</td>
<td>1.02 (0.32)</td>
</tr>
<tr>
<td>Dark Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 5-4. Comparisons of study parameters and photocatalysis half-lives among prior published studies. Asterisk indicates value was estimated based on graphical data provided.

<table>
<thead>
<tr>
<th>UV Source and Irradiance (if reported)</th>
<th>TiO₂ Design</th>
<th>Medium</th>
<th>Initial [MC] (µg/L)</th>
<th>Half-life (min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunlight (49-54 W/m² from 250-400nm)</td>
<td>Fixed-film</td>
<td>Pond H₂O</td>
<td>0.95 – 12.7</td>
<td>111-138</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>(batch reactor; not stirred)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8- 30W bulbs 254 nm</td>
<td>Slurry 0.2 g/L (recirculating system)</td>
<td>Distilled H₂O</td>
<td>55</td>
<td>4.2</td>
<td>Shepard et al. 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lake H₂O</td>
<td>64</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td>Xenon 280W UV Lamp 330-450 nm</td>
<td>Slurry 1% m/w (constantly stirred)</td>
<td>Milli-Q H₂O</td>
<td>200,000</td>
<td>2*</td>
<td>Lawton et al. 1999</td>
</tr>
<tr>
<td>Hg 100W Arc Lamp 365 nm (52 mw/cm²)</td>
<td>Slurry 1 g/L (constantly stirred)</td>
<td>Milli-Q H₂O</td>
<td>85</td>
<td>5*</td>
<td>Feitz et al. 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milli-Q H₂O</td>
<td>560</td>
<td>5.1*</td>
<td></td>
</tr>
<tr>
<td>Sunlight</td>
<td>Immobilized film (recirculating system)</td>
<td>Distilled H₂O</td>
<td>55</td>
<td>2.7</td>
<td>Shepard et al. 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lake H₂O</td>
<td>55</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Xenon 480W UV Lamp 330-450 nm</td>
<td>Slurry 1% m/v (constantly stirred)</td>
<td>Milli-Q H₂O</td>
<td>100,000-200,000</td>
<td>3.7**</td>
<td>Lawton et al. 2003</td>
</tr>
<tr>
<td>4- 15W bulbs (UV-A) 365 nm 717 W/m²</td>
<td>Fixed substrate: TiO₂ with graphene oxide (4% wt)</td>
<td>Milli-Q H₂O</td>
<td>10,000</td>
<td>4*</td>
<td>Fotiou et al. 2013</td>
</tr>
<tr>
<td>(at a distance of 25cm)</td>
<td>Slurry 0.2 g/L (Degussa P25)</td>
<td></td>
<td></td>
<td>2*</td>
<td></td>
</tr>
<tr>
<td>Simulated solar light: 150 W Xe Arc</td>
<td>Fixed substrate: TiO₂ with graphene oxide (4% wt)</td>
<td>Milli-Q H₂O</td>
<td>10,000</td>
<td>12*</td>
<td>Fotiou et al. 2013</td>
</tr>
<tr>
<td>Lamp 1150 W/m²</td>
<td>Slurry 0.2 g/L (Degussa P25)</td>
<td></td>
<td></td>
<td>6*</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5-1. Changes in total MC concentrations with cumulative UV insolation following sand filtration (n=3; error bars indicate ±1SD) for treatments and controls. Solid lines indicate pre- and post-sand filtration and dashed lines indicate concentrations with cumulative UV energy for photocatalysis.
CHAPTER SIX
SUMMARY AND CONCLUSIONS

The overall goal of this dissertation was to support progress in risk intervention for MC-producing cyanobacteria and MCs. To achieve this goal, a series of studies were conducted to provide a decision support system for risk intervention, and test hypotheses regarding specific management approaches that are targeted for MC-producing cyanobacteria (in-lake) and MCs in drinking water treatment processes (in-plant).

Cyanobacteria blooms occur rapidly and repeatedly in freshwater resources, creating urgent, high-pressure situations in which water resource managers must make critical decisions immediately. It would be useful to have the necessary data vetted and assembled logically such that a decision can be made logically, efficiently, and most importantly, based on best-available scientific data. The rationale for assembling information in this document began with the fundamental principle that exposures influence risks. Therefore, aspects of MC exposures were characterized in terms of source, chemical structures, environmental and toxicological properties, spatial and temporal distribution, and forms. Potential human exposure routes were characterized and ranked in terms of their important (i.e. routes more likely to result in significant exposures). Based on a strategic literature review with defined data acceptability criteria, data were 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 in mind, 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.
Based on those comparisons, the potential risks and financial losses associated with no-
action were clearly significant enough to warrant risk management for MC-producing
cyanobacteria (in-lake) as well as MCs (in-plant). Long-term and short-term risk
management approaches for MC-producing cyanobacteria and MCs were then reviewed
in terms of relative effectiveness, availability, durability, and scalability based on peer-
reviewed data. Finally, adaptive water resource management was described for this
context, and examples were provided for how that process could proceed. The goal of
this decision support system was to provide water resource managers, regulators, and
interested citizens and stakeholders with vetted and assembled information to aid in site-
specific decision making and development of adaptive water resource management plans.

Subsequent questions were asked regarding methods of altering MC exposures,
accomplished by targeting specific exposure-response relationships for a cyanobacterium
exposed to a copper-based algaecide and fate processes for MCs. In “Cell density
dependence of Microcystis aeruginosa responses to copper algaecide concentrations:
implications for microcystin-LR release” (Chapter Three), the influence of Microcystis
aeruginosa cell density on copper-based algaecide exposures and consequent responses
in terms of MC-LR release, were measured. Copper exposure concentrations strongly
correlated with copper doses for each cell density evaluated. Twenty-four-hour median
effect concentrations (24-h EC50s) for MC-LR release in terms of copper exposure
concentration ranged an order of magnitude with an order of magnitude increase in cell
density (0.03-0.3 mg Cu/L for 1x10^6 through 1x10^7 cells/mL). However, in terms of copper dose (sum mass of adsorbed and absorbed Cu/mg algae), 24-h EC50s were similar among all cell densities (0.005-0.006 mg Cu/mg algae). These laboratory physical models, combined with statistical models to estimate and compare EC50s, elucidated the significance of density-dependent exposures for copper-based algaecide applications. Combined with estimates of other site-specific factors (e.g. water characteristics) and processes (e.g. dilution and dispersion, sorption to organic matter and sediments), measurements of cell density prior to copper algaecide applications can refine predictions of in situ exposures and algal responses. Further, refining exposure-response predictions can decrease the likelihood of amending excessive or unnecessary copper concentrations to aquatic systems, as well as minimize risks for non-target aquatic organisms.

Following measurements of the density dependence of copper-based algaecide exposures and responses of cyanobacteria, questions remained regarding fate of MCs following copper algaecide exposures. In “Microcystin-LR degradation following copper-based algaecide exposures” (Chapter Four), the goal was to discern potential effects of decreases in dissolved oxygen concentrations on bacterial assemblages and MC degradation rates following copper-based algaecide exposures. As anticipated, cyanobacteria cell densities rapidly decreased within days following exposure to a copper-ethanolamine algaecide, and rates and extents of DO decline correlated with initial cyanobacteria cell densities. MC-LR degradation occurred at similar rates (half-lives 1 to 1.9-d), with the shortest half-life of 1-d occurring for the lowest cell density of cyanobacteria (1x10^6 cells/mL). Acinetobacter and Aeromonas were dominant bacteria.
genera in treatments following copper exposures, while these genera occupied minimal fractions of bacterial assemblages in untreated controls, indicating these genera may be able to utilize MCs for carbon and energy, especially in low DO (i.e. $< 2 \text{ mg L}^{-1}$) conditions. MC-LR half-lives measured in this study were comparable with measured half-lives in previous studies for a range of DO conditions. Further, relationships between cyanobacteria densities and MC-LR degradation half-lives demonstrated the benefits of intervening in early growth stages to minimize cyanobacteria densities and MC concentrations.

Finally, a study was conducted to evaluate a process that could be employed in the context of drinking water treatment for removal of MCs. In “Solar photocatalysis using fixed-film TiO$_2$ for microcystins from colonial *Microcystis aeruginosa*” (Chapter Five), rates of solar photocatalysis of MCs were measured using a fixed-film of TiO$_2$ preceded by sand filtration of waters with a range of cellular: aqueous MC ratios. Rates of photocatalysis were also calculated as a function of cumulative UV insolation. Finally, data were compared with previously published data for different experimental conditions. For water containing $< 10\%$ aqueous ($> 90\%$ cellular MC), sand filtration removed approximately 90% of total [MC], and the subsequent photocatalysis half-life averaged 0.35 MJ/m$^2$ (or 111-min). For the treatment consisting of $\sim 50\%$ aqueous MCs, sand filtration removed about 52% of total MCs, and the average half-life for photocatalysis was 0.37 MJ/m$^2$ (or 138-min). Finally, for the $> 90\%$ aqueous MC treatment, sand filtration removed 0% MCs, and the half-life for photocatalysis averaged 0.37 MJ/m$^2$ (or 135-min). Results from this study are likely representative of treatment
scenarios with waters containing confounding characteristics for treatment (i.e. turbidity and DOC) and sunlight as the UV source, which is more likely in developing countries with less advanced (i.e. fossil fuel energy demanding methods) for drinking water treatment. Photocatalysis is a rapid and effective process for decreasing concentrations of MCs, and could be useful for mitigating risks from MC exposures in drinking water resources.

Clearly, cyanobacteria that produce MCs will be a legacy issue in freshwater resources worldwide. In the near future, it will likely become clear that “no-action” is no longer an acceptable decision. In order to maintain designated uses of water resources and minimize potential for risks to humans and other biota, environmentally sound, economically feasible, and socially acceptable means of risk management are necessary. Collectively, the experiments in this dissertation contributed data that can be used to support scientifically-driven risk intervention for MCs produced by cyanobacteria in critical freshwater resources. Clearly, there are remaining data gaps, particularly in the area of human health. However, there is enough information available to manage risks 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.

Contributions to scientific literature

Chapter Two: Targeted submission to Environmental Monitoring and Assessment
Chapter Three:

Chapter Four:

Chapter Five: