

# Associations between Chlorophyll *a* and various Microcystin-LR Health Advisory Concentrations

Jeffrey W. Hollister<sup>\*</sup> <sup>1</sup> Betty J. Kreakie<sup>1</sup>

<sup>1</sup>US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive Narragansett, RI, 02882, USA

<sup>\*</sup> corresponding author: [hollister.jeff@epa.gov](mailto:hollister.jeff@epa.gov)

Cyanobacteria harmful algal blooms (cHABs) are associated with a wide range of adverse health effects that stem mostly from the presence of cyanotoxins. To help protect against these impacts, several health advisory levels have been set for some toxins. In particular, one of the more common toxins, microcystin, has several advisory levels set for drinking water and recreational use. However, compared to other water quality measures, field measurements of microcystin are not commonly available due to cost and advanced understanding required to interpret results. Addressing these issues will take time and resources. Thus, there is utility in finding indicators of microcystin that are already widely available, can be estimated quickly and *in situ*, and used as a first defense against high levels of microcystin. Chlorophyll *a* is commonly measured, can be estimated *in situ*, and has been shown to be positively associated with microcystin. In this paper, we use this association to provide estimates of chlorophyll *a* concentrations that are indicative of a higher probability of exceeding select health advisory concentrations for microcystin-LR. Using the 2007 National Lakes Assessment and a conditional probability approach, we identify chlorophyll *a* concentrations that are more likely than not to be associated with an exceedance of a microcystin health advisory level. We look at the recent US EPA health advisories for drinking water as well as the World Health Organization levels for drinking water and recreational use and identify a range of chlorophyll *a* thresholds. A 50% chance of exceeding one of the specific advisory microcystin concentrations of 0.3, 1, 1.6, and 2  $\mu\text{g/L}$  is associated with chlorophyll *a* concentration thresholds of 20.88, 69.6, 84.24, and 97.49, respectively. When managing for these various microcystin levels, exceeding these reported chlorophyll *a* concentrations should be a trigger for further testing and possible management action.

## 1 Introduction

Over the last decade, numerous events and legislative activities have raised the public awareness of harmful algal blooms (Rinta-Kanto et al. 2009, HABHRCA 2014, Jetoo et al. 2015). In response the US Environmental Protection Agency (USEPA) has recently released suggested microcystin-LR (one of the more common toxins) concentrations that would trigger health advisories (McElhiney and Lawton 2005, Zurawell et al. 2005, USEPA 2015). Additionally, the World Health Organization (WHO) has proposed microcystin advisory levels for drinking water and a range of recreational risk levels (Chorus

36 and Bartram 1999, Organization 2003). While these levels and associated advisories are likely to help  
37 mitigate the impacts from harmful algal blooms, they are not without complications.

38 One of these complications is that they rely on available measurements of microcystin-LR. While  
39 laboratory testing (e.g., chromatography) remains the gold standard for quantifying microcystin-LR  
40 concentrations in water samples, several field test kits have been developed. Even though field tests  
41 provide a much needed means for rapid assessment, they are not yet widely used and are moderately  
42 expensive (approximately \$150-\$200 depending on specific kit) with a limited shelf life (typically one  
43 year) (James et al. 2011, Aranda-Rodriguez et al. 2015). Additionally, each technique requires nuanced  
44 understanding of the detection method (e.g., limit of detection, specific microcystin variants being  
45 measured, and sampling protocol).

46 Fortunately, microcystin-LR has been shown to be associated with several other, more commonly  
47 measured and well understood components of water quality that are readily assessed in the field. For  
48 instance, there are small or hand held fluorometers that measure chlorophyll *a*. Additionally, chlorophyll  
49 *a* is a very commonly measured component of water quality that is also known to be positively associated  
50 with microcystin-LR concentrations (Pip and Bowman 2014, Yuan et al. 2014). Yuan et. al (2014)  
51 explore these associations in detail and control for other related variables. In their analysis they find  
52 that total nitrogen and chlorophyll *a* show the strongest association with microcystin. Furthermore,  
53 they identify chlorophyll *a* and total nitrogen concentrations that are associated with exceeding 1  $\mu\text{g/L}$  of  
54 microcystin. Given these facts, it should be possible to identify chlorophyll *a* concentrations that would  
55 be associated with the new USEPA Microcystin-LR health advisory levels for drinking water. Identifying  
56 these associations would provide another tool for water resource managers to help manage the threat  
57 to public health posed by CHABs and would be especially useful in the absence of microcystin-LR  
58 concentrations.

59 In this paper we build on past efforts and utilize the National Lakes Assessment (NLA) data and  
60 identify chlorophyll *a* concentrations that are associated with higher probabilities of exceeding several  
61 microcystin-LR health advisory concentrations (Chorus and Bartram 1999, USEPA 2009, 2015). We  
62 add to past studies by exploring associations with newly announced advisory levels and by also  
63 applying a different method, conditional probability analysis. Utilizing different methods strengthens

the evidence for suggested chlorophyll *a* levels that are associated with increased risk of exceeding the health advisory levels as those levels are not predicated on a single analytical method. So that others may repeat or adjust this analysis, the data, code, and this manuscript are freely available via <https://github.com/USAPE/microcystinchla>.

## 2 Methods

### 2.1 Data

We used the 2007 NLA chlorophyll *a* and microcystin-LR concentration data (USEPA 2009, NLA). These data represent a snapshot of water quality from the summer of 2007 for the conterminous United States and were collected as part of an ongoing probabilistic monitoring program (USEPA 2009). Data on chlorophyll *a* and microcystin-LR concentrations are available for lakes.

### 2.2 Conditional Probability Analysis

We used a conditional probability analysis (CPA) approach to explore associations between chlorophyll *a* concentrations and World Health Organization (WHO) and USEPA microcystin-LR health advisory levels (Paul and Munns 2011). Many levels have been suggested (Table 1), but lakes with higher microcystin-LR concentrations in the NLA were rare. Only 1.16 % of lakes sampled had a concentration greater than 10. Thus, for this analysis we focus on the microcystin concentrations that are better represented in the NLA data. These were the USEPA children’s drinking water advisory level of 0.3  $\mu\text{g/L}$  (USEPA Child), the WHO drinking water advisory level of 1  $\mu\text{g/L}$  (WHO Drinking), the USEPA adult drinking water advisory level of 1.6  $\mu\text{g/L}$  (USEPA Adult), and the WHO recreational, low probability of effect advisory level of 2  $\mu\text{g/L}$  (WHO Recreational).

Conditional probability analysis provides information about the probability of observing one event given another event has also occurred. For this analysis, we used CPA to examine how the conditional probability of exceeding one of the health advisories changes as chlorophyll *a* increases in a lake. We expect to find higher chlorophyll *a* concentrations to be associated with higher probabilities of exceeding

the microcystin-LR health advisory levels. We also calculated bootstrapped 95% confidence intervals (CI) using 1000 bootstrapped samples. Thus, to identify chlorophyll *a* concentrations of concern we identify the value of the upper 95% CI across a range of conditional probabilities of exceeding each health advisory level. Using the upper confidence limit to identify a threshold is justified as it ensures that a given threshold is unlikely to miss a microcystin exceedance.

As both microcystin-LR and chlorophyll *a* values were highly skewed right, a log base 10 transformation was used. Additional details of the specific implementation are available at <https://github.com/USEPA/microcystinchla>. A more detailed discussion of CPA is beyond the scope of this paper, but see Paul et al. (2005) and Hollister et al. (2008) for greater detail. Lastly, all analyses were conducted using R version 3.2.2 and code and data from this analysis are freely available as an R package at <https://github.com/USAPE/microcystinchla>.

### 3 Results

In the 2007 NLA, microcystin-LR concentrations ranged from 0.05 to 225  $\mu\text{g/L}$ . Microcystin-LR concentrations of 0.05  $\mu\text{g/L}$  represent the detection limits. Any value greater than that indicates the presence of microcystin-LR. Of those lakes with microcystin, the median concentration was 0.51 and the mean was 3.17. Of all lakes sampled, 21% of lakes exceeded the USEPA Child level, 8.8% of lakes exceeded the USEPA Adult level, 11.7% of lakes exceeded the WHO Drinking level, and 7.3% of lakes exceeded the WHO Recreational level. For chlorophyll *a*, the range was 0.07 to 936  $\mu\text{g/L}$ . All lakes had reported chlorophyll *a* concentrations that exceeded detection limits. The median concentration was 7.79  $\mu\text{g/L}$  and the mean was 29.63  $\mu\text{g/L}$ . The associations between chlorophyll *a* and the upper confidence interval across a range of conditional probability values is shown in Table 2. Specific chlorophyll *a* that are associated with greater than even odds of exceeding the advisory levels were 0.07, 0.07, 2.89, and 10.27 for 0.3, 1.0, 1.6 and 2.0  $\mu\text{g/L}$  advisory levels, respectively (Table 2 & Figure 2).

## 111 4 Discussion

112 The association between Log10 microcystin-LR and Log10 chlorophyll *a* shows a wedge pattern (Figure  
113 1). This indicates that, in general, higher concentrations of microcystin-LR almost always co-occur with  
114 higher concentrations of chlorophyll *a* yet the inverse is not true. Higher chlorophyll *a* is not necessarily  
115 predictive of higher microcystin-LR concentrations; however, chlorophyll *a* may be predictive of the  
116 probability of exceeding a certain threshold.

117 This is the case as the probability of exceeding each of the four tested health advisory levels increases  
118 as a function of chlorophyll *a* concentration (Figure 2). We use this association to identify chlorophyll *a*  
119 concentrations that are associated with a range of probabilities of exceeding a given health advisory  
120 level (Table 2). For the purposes of this discussion we focus on a conditional probability of 50% or  
121 greater (i.e. greater than even odds to exceed a health advisory level). The 50% conditional probability  
122 chlorophyll *a* thresholds represents 30.1%, 10.6%, 8.8%, and 7.9% of sample lakes for the USEPA Child,  
123 the WHO Drinking, the USEPA Adult, and the WHO recreational levels, respectively.

124 Furthermore, the chlorophyll *a* cutoffs may be used to predict whether or not a lake exceeds the  
125 microcystin-LR health advisories. Doing so allows us to compare the accuracy of the prediction as well  
126 as evaluate false negatives. Total accuracy of the four cutoffs predicting microcystin-LR exceedances  
127 were 74% for the USEPA children’s advisory, 86% for the WHO drinking water advisory, 89% for the  
128 USEPA adult advisory and, 90% for the WHO recreational advisory (Tables 3, 4, 5, & 6). However,  
129 total accuracy is only one part of the prediction performance with which we are concerned.

130 When using the chlorophyll *a* cutoffs as an indicator of microcystin-LR exceedances, the error that  
131 should be avoided is predicting that no exceedance has occurred when in fact it has. In other words,  
132 we would like to avoid Type II errors and minimize the proportion of false negatives. For the four  
133 chlorophyll *a* cut-offs we had a proportion of false negatives of 9%, 8%, 6% and , 5% for the U.S EPA  
134 childrens drinking water, the WHO drinking water, the USEPA adult drinking water, and the WHO  
135 recreational advisories, respectively. In each case we miss less than 10% of the lakes that are in fact  
136 exceeding the microcystin-LR advisory. While this method performs well with regard to the false  
137 negative percentag, it is possible that is a relic of the NLA dataset and testing with additional data

138 should be explore to further substantiate this result.

139 There are numerous possible uses for the chlorophyll *a* and microcystin-LR advisory cut-off values.  
140 First, in the absence of microcystin-LR measurements, exceedence of the chlorophyll *a* concentrations  
141 could be a trigger for further actions. Given that there is uncertainty around these chlorophyll *a* cutoffs  
142 the best case scenario would be to monitor for chlorophyll *a* and in the event of exceeding a target  
143 concentration take water samples and have those samples tested in a lab for microcystin-LR.

144 A second potential use is to identify past bloom events from historical data. As harmful algal blooms  
145 are made up of many species and have various mechanisms responsible for adverse impacts (e.g. toxins,  
146 hypoxia, odors), there is no single definition of a bloom. For cHABs one approach has been to identify  
147 an increase over a baseline concentration of phycocyanin (Miller et al. 2013). This is a useful approach  
148 for targeted studies, but phycocyanin is also not always available and measures the predominance of  
149 cyanobacterial pigments and not toxins. Using our chlorophyll *a* cutoffs provides a value that is more  
150 directly associated with microcystin-LR and can be used to classify lakes, from past surveys, as having  
151 bloomed.

152 Lastly, using chlorophyll *a* is not meant as a replacement for testing of microcystin-LR or other toxins.  
153 It should be used when other, direct measurements of cyanotoxins are not available. In those cases,  
154 which are likely to be common at least in the near future, using a more ubiquitous measurement, such as  
155 chlorophyll *a* will provide a reasonable proxy for the probability of exceeding a microcystin-LR health  
156 advisory level and provide better protection against adverse effects in both drinking and recreational  
157 use cases.

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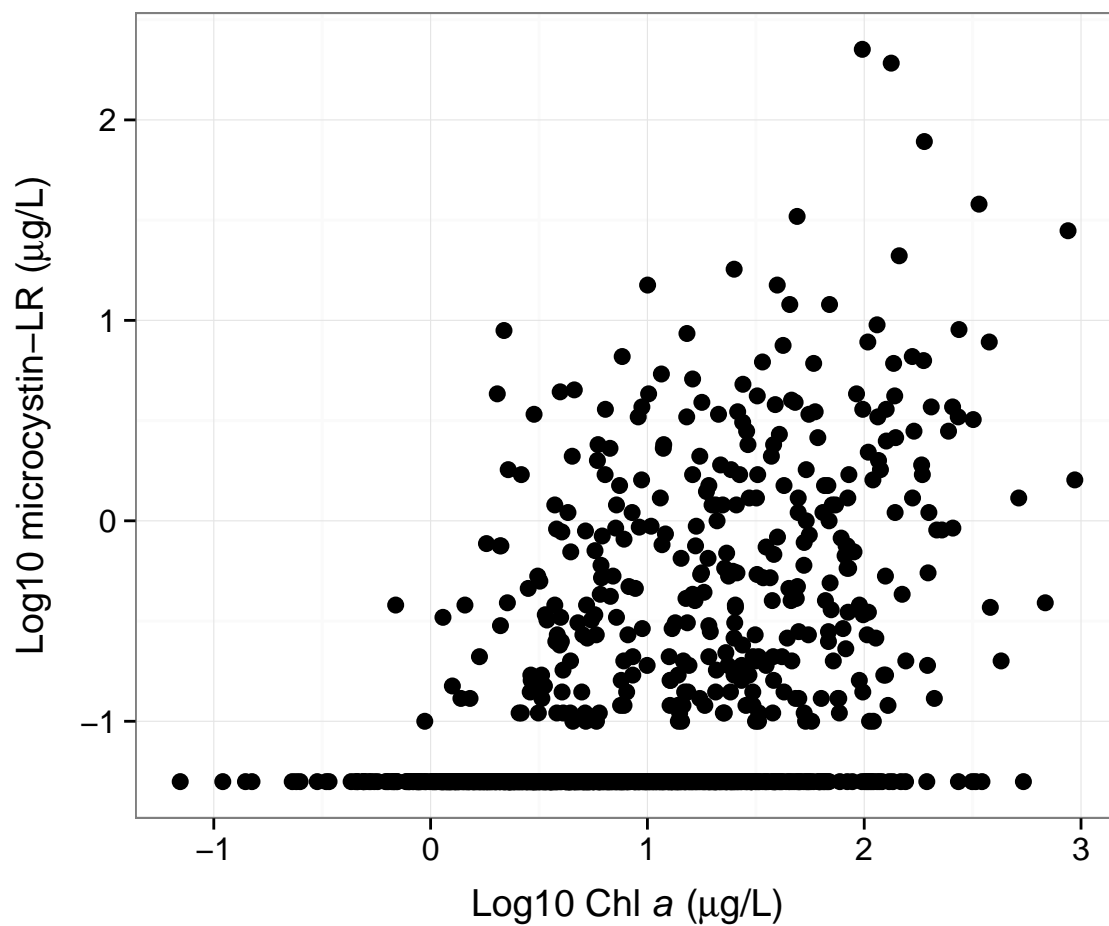


Figure 1: Scatterplot showing association between chlorophyll *a* and microcystin-LR.



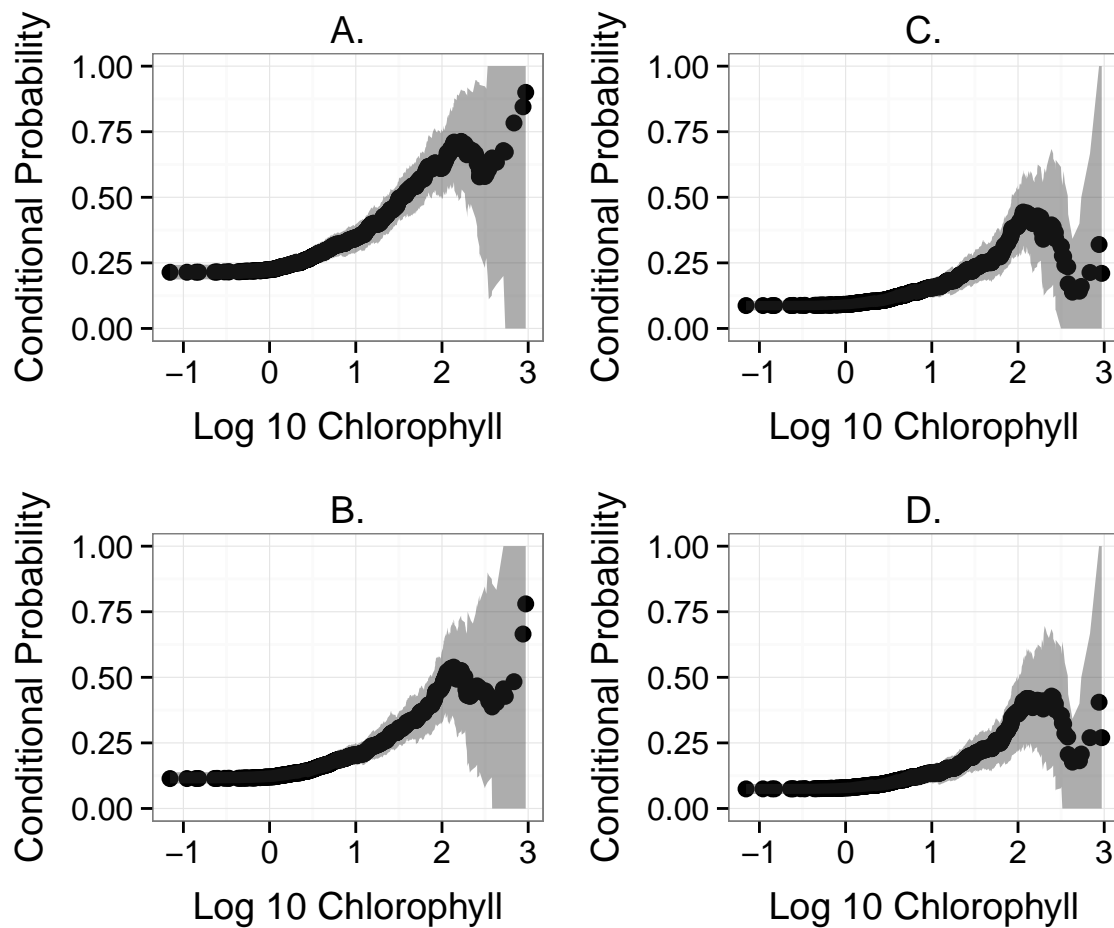


Figure 2: Conditional probability plots showing association between the probability of exceeding various microcystin-LR (MLR) health advisory Levels. A.) Plot for USEPA Child (0.3  $\mu\text{g/L}$ ). B.) Plot for WHO Drinking (1  $\mu\text{g/L}$ ). C.) Plot for USEPA Adult (1.6  $\mu\text{g/L}$ ). D.) Plot for WHO Recreational (2  $\mu\text{g/L}$ ).

Table 1: Various suggested microcystin-LR health advisory concentrations.

Source	Type	Concentration
USEPA	Adult Drinking Water Advisory	1.6 $\mu\text{g/L}$
USEPA	Child Drinking Water Advisory	0.3 $\mu\text{g/L}$
WHO	Drinking Water	1 $\mu\text{g/L}$
WHO	Recreational: High Prob. of Effect	20-2000 $\mu\text{g/L}$
WHO	Recreational: Low Prob. of Effect	2-4 $\mu\text{g/L}$
WHO	Recreational: Moderate Prob. of Effect	10-20 $\mu\text{g/L}$
WHO	Recreational: Very High Prob. of Effect	>2000 $\mu\text{g/L}$

Table 2: Chlorophyll *a* concentrations that are associated with a 50% probability of exceeding a microcystin-LR health advisory concentration.

Cond. Probability	USEPA Child	WHO Drink	USEPA Adult	WHO Recreational
0.1	0.07	0.07	0.07	1.17
0.2	0.07	4.38	11.84	16.80
0.3	2.89	17.49	32.11	45.22
0.4	10.27	38.30	66.96	76.80
0.5	20.88	69.60	84.24	97.49
0.6	38.20	100.51	114.62	125.40
0.7	65.60	128.74	871.20	871.20
0.8	114.62	271.44	871.20	871.20
0.9	166.63	338.40	871.20	871.20

Table 3: Confusion matrix comparing chlorophyll *a* predicted exceedences (rows) versus real exceedences (columns) for the USEPA childrens drinking water advisory.

	FALSE	TRUE
FALSE	632	90
TRUE	179	127

Table 4: Confusion matrix comparing chlorophyll *a* predicted exceedences (rows) versus real exceedences (columns) for the WHO drinking water advisory.

	FALSE	TRUE
FALSE	844	80
TRUE	63	41

Table 5: Confusion matrix comparing chlorophyll *a* predicted exceedences (rows) versus real exceedences (columns) for the USEPA adult drinking water advisory.

	FALSE	TRUE
FALSE	885	57
TRUE	52	34

Table 6: Confusion matrix comparing chlorophyll *a* predicted exceedences (rows) versus real exceedences (columns) for the WHO recreational water advisory.

	FALSE	TRUE
FALSE	903	48
TRUE	50	27

## References

- Aranda-Rodriguez, R., Z. Jin, J. Harvie, and A. Cabecinha. 2015. Evaluation of three field test kits to detect microcystins from a public health perspective. *Harmful Algae* 42:34–42.
- Chorus, E. I., and J. Bartram. 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management.
- HABHRCA. 2014. Harmful algal bloom and hypoxia research and control amendments act of 2014.
- Hollister, J. W., H. A. Walker, and J. F. Paul. 2008. CProb: A computational tool for conducting conditional probability analysis. *Journal of environmental quality* 37:2392–2396.
- James, R., A. Gregg, A. Dindal, and J. McKernan. 2011. Environmental technology verification report: Abraxis microcystin test kits. Online document. Accessed online: June 22.
- Jetoo, S., V. I. Grover, and G. Krantzberg. 2015. The toledo drinking water advisory: Suggested application of the water safety planning approach. *Sustainability* 7:9787–9808.
- McElhiney, J., and L. A. Lawton. 2005. Detection of the cyanobacterial hepatotoxins microcystins. *Toxicology and Applied Pharmacology* 203:219–230.
- Miller, T. R., L. Beversdorf, S. D. Chaston, and K. D. McMahon. 2013. Spatiotemporal molecular analysis of cyanobacteria blooms reveals microcystis-aphanizomenon interactions. *PloS one* 8:e74933.
- Organization, W. H. 2003. Cyanobacterial toxins: Microcystin-LR in drinking-water. background document for development of WHO guidelines for drinking-water quality. geneva, switzerland. World Health Organization, 2nd ed. Geneva.
- Paul, J. F., and M. E. McDonald. 2005. Development of empirical, geographically specific water quality criteria: A conditional probability analysis approach 41:1211–1223.
- Paul, J. F., and W. R. Munns. 2011. Probability surveys, conditional probability, and ecological risk assessment. *Environmental Toxicology and Chemistry* 30:1488–1495.



191 Pip, E., and L. Bowman. 2014. Microcystin and algal chlorophyll in relation to nearshore nutrient  
192 concentrations in lake winnipeg, canada. *Environment and Pollution* 3:p36.

193 Rinta-Kanto, J. M., E. A. Konopko, J. M. DeBruyn, R. A. Bourbonniere, G. L. Boyer, and S. W.  
194 Wilhelm. 2009. Lake erie microcystis: Relationship between microcystin production, dynamics of  
195 genotypes and environmental parameters in a large lake. *Harmful Algae* 8:665–673.

196 USEPA. 2009. National lakes assessment: A collaborative survey of the nation’s lakes. ePA 841-r-09-001.  
197 Office of Water; Office of Research; Development, US Environmental Protection Agency Washington,  
198 DC.

199 USEPA. 2015. Drinking water health advisory for the cyanobacterial microcystin toxins. ePA-820R15100.  
200 Office of Water, US Environmental Protection Agency Washington, DC.

201 Yuan, L. L., A. I. Pollard, S. Pather, J. L. Oliver, and L. D’Anglada. 2014. Managing microcystin:  
202 Identifying national-scale thresholds for total nitrogen and chlorophyll a. *Freshwater Biology* 59:1970–  
203 1981.

204 Zurawell, R. W., H. Chen, J. M. Burke, and E. E. Prepas. 2005. Hepatotoxic cyanobacteria: A review  
205 of the biological importance of microcystins in freshwater environments. *Journal of Toxicology and*  
206 *Environmental Health, Part B* 8:1–37.