

# Associations between chlorophyll *a* and various microcystin-LR health advisory Concentrations

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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 21.92, 77.2, 84.96, and 105.84, 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 [1–3]. 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 [4–6]. Additionally, the World Health Organization (WHO) has proposed microcystin advisory levels for drinking water and a range of recreational risk levels [7,8]. While these levels and associated advisories are likely to help mitigate the impacts from harmful algal blooms, they are not

36 without complications.

37 One of these complications is that they rely on available measurements of microcystin-LR. While  
38 laboratory testing (e.g., chromatography) remains the gold standard for quantifying microcystin-LR  
39 concentrations in water samples, several field test kits have been developed. Even though field tests  
40 provide a much needed means for rapid assessment, they are not yet widely used and are moderately  
41 expensive (approximately \$150-\$200 depending on specific kit) with a limited shelf life (typically one  
42 year) [9,10]. Additionally, each technique requires nuanced understanding of the detection method (e.g.,  
43 limit of detection, specific microcystin variants being measured, and sampling protocol).

44 Fortunately, microcystin-LR has been shown to be associated with several other, more commonly  
45 measured and well understood components of water quality that are readily assessed in the field. For  
46 instance, there are small or hand held fluorometers that measure chlorophyll *a*. Additionally, chlorophyll  
47 *a* is a very commonly measured component of water quality that is also known to be positively associated  
48 with microcystin-LR concentrations [11,12]. Yuan et. al [12] explore these associations in detail and  
49 control for other related variables. In their analysis they find that total nitrogen and chlorophyll *a* show  
50 the strongest association with microcystin. Furthermore, they identify chlorophyll *a* and total nitrogen  
51 concentrations that are associated with exceeding 1  $\mu\text{g/L}$  of microcystin. Given these facts, it should  
52 be possible to identify chlorophyll *a* concentrations that would be associated with the new USEPA  
53 microcystin-LR health advisory levels for drinking water. Identifying these associations would provide  
54 another tool for water resource managers to help manage the threat to public health posed by cHABs  
55 and would be especially useful in the absence of measured microcystin-LR concentrations.

56 In this paper we build on past efforts and utilize the National Lakes Assessment (NLA) data and  
57 identify chlorophyll *a* concentrations that are associated with higher probabilities of exceeding several  
58 microcystin-LR health advisory concentrations [6,8,13]. We add to past studies by exploring associations  
59 with newly announced advisory levels and by also applying a different method, conditional probability  
60 analysis. Utilizing different methods strengthens the evidence for suggested chlorophyll *a* levels that are  
61 associated with increased risk of exceeding the health advisory levels as those levels are not predicated  
62 on a single analytical method. So that others may repeat or adjust this analysis, the data, code, and  
63 this manuscript are freely available via <https://github.com/USEPA/microcystinchla>.

## 2 Methods

### 2.1 Data

We used the 2007 NLA chlorophyll *a* and microcystin-LR concentration data [13]. 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 [13]. Data on chlorophyll *a* and microcystin-LR concentrations are available for lakes.

### 2.2 Analytical Methods

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 [14]. Many health advisory 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  $\mu\text{g/L}$ . Thus, for this analysis we focused 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.

89 As both microcystin-LR and chlorophyll *a* values were highly skewed right, a log base 10 transformation  
90 was used. Additional details of the specific implementation are available at [https://github.com/](https://github.com/USEPA/microcystinchla)  
91 [USEPA/microcystinchla](https://github.com/USEPA/microcystinchla). A more detailed discussion of CPA is beyond the scope of this paper, but  
92 see Paul et al. [15] and Hollister et al. [16] for greater detail. Lastly, all analyses were conducted  
93 using R version 3.2.2 and code and data from this analysis are freely available as an R package at  
94 <https://github.com/USEPA/microcystinchla>.

95 Lastly, we assess the ability of these chlorophyll *a* thresholds to predict microcystin exceedance. We use  
96 error matrices and calculate total accuracy as well as the proportion of false negatives. Total accuracy is  
97 the total number of correct predictions divided by total observations. The proportion of false negatives  
98 is the total number of lakes that were predicted to not exceed the microcystin guidelines but actually  
99 did, divided by the total number of observations.

### 100 3 Results

101 In the 2007 NLA, microcystin-LR concentrations ranged from 0.05 to 225  $\mu\text{g/L}$ . Microcystin-LR  
102 concentrations of 0.05  $\mu\text{g/L}$  represent the detection limits. Any value greater than that indicates the  
103 presence of microcystin-LR. Of those lakes with microcystin, the median concentration was 0.51  $\mu\text{g/L}$   
104 and the mean was 3.17  $\mu\text{g/L}$ . Of all lakes sampled, 21% of lakes exceeded the USEPA Child level, 8.8%  
105 of lakes exceeded the USEPA Adult level, 11.7% of lakes exceeded the WHO Drinking level, and 7.3% of  
106 lakes exceeded the WHO Recreational level. For chlorophyll *a*, the range was 0.07 to 936  $\mu\text{g/L}$ . All lakes  
107 had reported chlorophyll *a* concentrations that exceeded detection limits. The median concentration  
108 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  
109 confidence interval across a range of conditional probability values are shown in Table 2. Specific  
110 chlorophyll *a* that are associated with greater than even odds of exceeding the advisory levels were 0.07,  
111 0.07, 2.78, and 9.97  $\mu\text{g/L}$  for 0.3, 1.0, 1.6, and 2.0  $\mu\text{g/L}$  advisory levels, respectively (Table 2 & Figure  
112 2).

113 The chlorophyll *a* cutoffs may be used to predict whether or not a lake exceeds the microcystin-LR  
114 health advisories. Doing so allows us to compare the accuracy of the prediction as well as evaluate

115 false negatives. Total accuracy of the four cutoffs predicting microcystin-LR exceedances were 19% for  
116 the USEPA children’s advisory, 22% for the WHO drinking water advisory, 22% for the USEPA adult  
117 advisory, and 23% for the WHO recreational advisory (Tables 3, 4, 5, & 6). However, total accuracy is  
118 only one part of the prediction performance with which we are concerned.

119 When using the chlorophyll *a* cutoffs as an indicator of microcystin-LR exceedances, the error that  
120 should be avoided is predicting that no exceedance has occurred when in fact it has. In other words, we  
121 would like to avoid Type II errors and minimize the proportion of false negatives. For the four chlorophyll  
122 *a* cut-offs we had a proportion of false negatives of 2%, 2%, 1%, and 1% for the USEPA children’s, the  
123 WHO drinking water, the USEPA adult, and the WHO recreational advisories, respectively. In each  
124 case we missed less than 10% of the lakes that in fact exceeded the microcystin-LR advisory. While this  
125 method performs well with regard to the false negative percentage, it is possible that is a relic of the  
126 NLA dataset and testing with additional data would allow us to confirm this result.

## 127 4 Discussion

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

133 This is the case as the probability of exceeding each of the four tested health advisory levels increases  
134 as a function of chlorophyll *a* concentration (Figure 2). We used this association to identify chlorophyll  
135 *a* concentrations that are associated with a range of probabilities of exceeding a given health advisory  
136 level (Table 2). For the purposes of this discussion we focus on a conditional probability of 50% or  
137 greater (i.e., greater than even odds to exceed a health advisory level). The 50% conditional probability  
138 chlorophyll *a* thresholds represents 29.2%, 9.8%, 8.6%, and 6.9% of sample lakes for the USEPA Child,  
139 the WHO Drinking, the USEPA Adult, and the WHO recreational levels, respectively.

140 There are numerous possible uses for the chlorophyll *a* and microcystin-LR advisory cut-off values.

141 First, in the absence of microcystin-LR measurements, exceedence of the chlorophyll *a* concentrations  
142 could be a trigger for further actions. Given that there is uncertainty around these chlorophyll *a* cutoffs  
143 the best case scenario would be to monitor for chlorophyll *a* and in the event of exceeding a target  
144 concentration take water samples and have those samples tested for microcystin-LR.

145 A second potential use is to identify past bloom events from historical data. As harmful algal blooms  
146 are made up of many species and have various mechanisms responsible for adverse impacts (e.g., toxins,  
147 hypoxia, odors), there is no single definition of a bloom. For cHABs, one approach has been to identify  
148 an increase over a baseline concentration of phycocyanin [17]. This is a useful approach for targeted  
149 studies, but phycocyanin is also not always available and measures the predominance of cyanobacterial  
150 pigments and not toxins. Using our chlorophyll *a* cutoffs provides a value that is more directly associated  
151 with microcystin-LR and can be used to classify lakes, from past surveys, as having 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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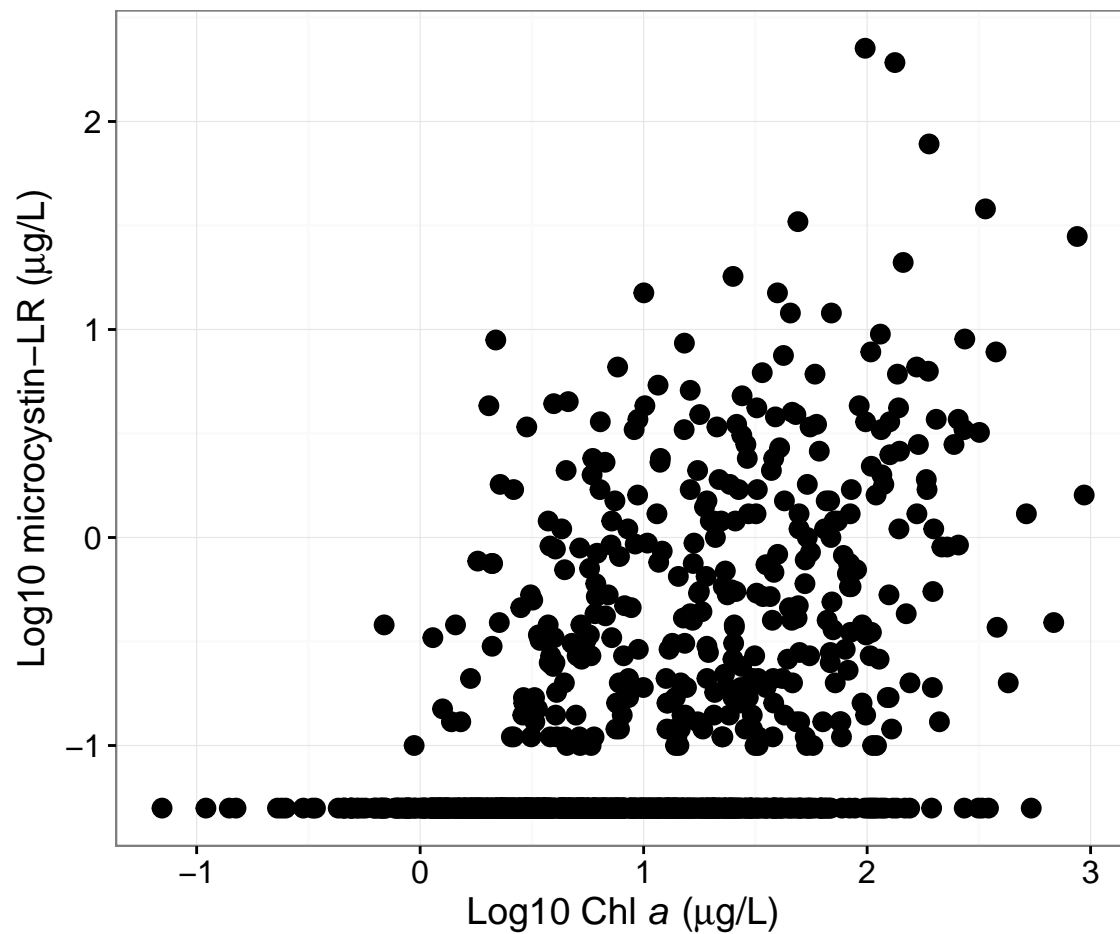


Figure 1: Scatterplot showing association between chlorophyll *a* and microcystin-LR.

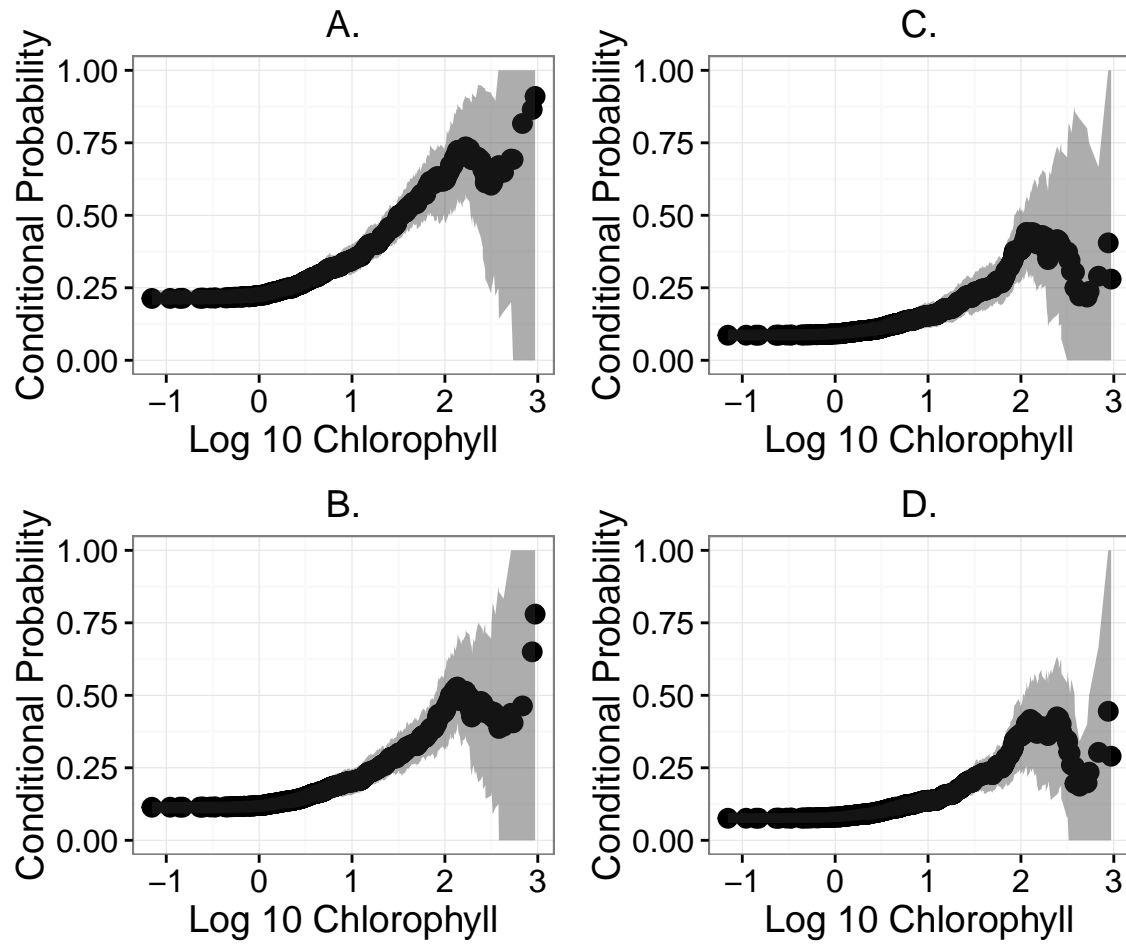


Figure 2: Conditional probability plots showing association between the probability of exceeding various microcystin-LR (MLR) health advisory Levels. A.) Plot for USEPA Child ( $0.3 \mu\text{g/L}$ ). B.) Plot for WHO Drinking ( $1 \mu\text{g/L}$ ). C.) Plot for USEPA Adult ( $1.6 \mu\text{g/L}$ ). D.) 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        | 4.48      | 10.67       | 18.24            |
| 0.3               | 2.78        | 17.49     | 31.59       | 50.26            |
| 0.4               | 9.97        | 38.73     | 69.19       | 76.80            |
| 0.5               | 21.92       | 77.20     | 84.96       | 105.84           |
| 0.6               | 38.73       | 103.20    | 133.20      | 228.24           |
| 0.7               | 63.94       | 138.24    | 244.80      | 871.20           |
| 0.8               | 113.14      | 326.88    | 326.88      | 871.20           |
| 0.9               | 166.63      | 516.00    | 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.

|               | Not Exceed | Exceed | Row Totals |
|---------------|------------|--------|------------|
| Not Exceed    | 638        | 93     | 731        |
| Exceed        | 173        | 124    | 297        |
| Column Totals | 811        | 217    | 1028       |

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

|               | Not Exceed | Exceed | Row Totals |
|---------------|------------|--------|------------|
| Not Exceed    | 850        | 82     | 932        |
| Exceed        | 57         | 39     | 96         |
| Column Totals | 907        | 121    | 1028       |

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

|               | Not Exceed | Exceed | Row Totals |
|---------------|------------|--------|------------|
| Not Exceed    | 887        | 57     | 944        |
| Exceed        | 50         | 34     | 84         |
| Column Totals | 937        | 91     | 1028       |

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

|               | Not Exceed | Exceed | Row Totals |
|---------------|------------|--------|------------|
| Not Exceed    | 910        | 52     | 962        |
| Exceed        | 43         | 23     | 66         |
| Column Totals | 953        | 75     | 1028       |

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