California Regional Water Quality Control Board
Colorado River Basin Region
Surface Water Ambient Monitoring Program Harmful Algal Blooms Program

Assessment of Cyanobacteria and Cyanotoxins in the Colorado River from Lake Havasu to the Bill Williams Wildlife Preserve

2023 Summary Report

ACKNOWLEDGEMENTS

The Colorado River Basin Regional Water Quality Control Board would like to acknowledge Chase Ehlo from the U.S. Fish and Wildlife Service, Arizona, for his excellent assistance that made this assessment possible.

The Regional Water Board also wishes to thank student assistant Lauren Haugen of Lake Havasu City for her outstanding contribution and dedication to this assessment.

Finally, the Regional Water Board would like to thank Marisa VanDyke of the State Water Resources Control Board for her assistance in getting this assessment funded.

CONTENTS

Assessment of Cyanobacteria and Cyanotoxins in the Colorado River from Lake Havasu to the Bill Williams Wildlife Preserve

Surface Water Ambient Monitoring Program
Harmful Algal Blooms Program
California Regional Water Quality Control Board
Colorado River Basin Region -June 2023

Abstract

In September and October of 2022, the California Regional Water Quality Control Board, Colorado River Basin assessed the occurrence and distribution of cyanobacteria and associated cyanotoxins along a lower reach of the Colorado River. The purpose of the assessment was to determine if cyanobacteria was widespread in the river and to quantify cyanotoxin concentrations. Under the Surface Water Ambient Monitoring Program's (SWAMP) Harmful Algal Blooms (HABs) program, water grab samples were collected from thirty sites along the Colorado River, between Lake Havasu and the Bill Williams Wildlife Preserve. Samples were analyzed using Enzyme Linked Immunosorbent Assays (ELISA). Cyanotoxins were detected in twenty-four of the thirty water samples collected (eighty-percent), with eight of those samples triggering the issuance of public health warnings; four samples exceeded the 'danger' level protocol threshold of 20 µg/L, one sample exceeded the 'warning' level threshold of 6.0 µg/L, and three samples exceeded the 'caution' level threshold of 0.8 µg/L. Various densities of confirmed M*icrocystis* spp. colonies were observed floating on the water's surface and suspended in the water column at several of the sampling sites, while microcystin was the most frequently detected cyanotoxin. The highest concentration of microcystin detected during this assessment measured 2,606 µg/L, which is 130-times the danger-level threshold of 20 µg/L. That detection was made at Lake Havasu State Park, in the vicinity of the personal watercraft launch ramp where body contact with the water is frequent. Based on these findings, additional HABs monitoring at Lake Havasu and the lower Colorado River is strongly recommended for the protection of public and environmental health.

Background

The Colorado River is greatly understudied in terms of Harmful Algal Blooms (HABs), and there is limited monitoring data available from regulatory and non-regulatory agencies located in both California and Arizona for assessing the extent of HABs in the river. This assessment marks the first time that the Colorado River Basin Regional Water Board has monitored HABs on the river, made possible by the availability of additional funding. And while agencies regularly receive funding for monitoring waterbodies, those agencies must determine which waterbodies and analytes are priority, based on available funding.

Both the river and Lake Havasu are heavily used for recreation, and the Lake Havasu region alone attracts more than 830,000 visitors annually. Cyanotoxins are known to be harmful or even fatal to both people and animals, and sudden blooms can produce dangerous concentrations of these toxins. Characterizing cyanobacteria and associated cyanotoxins along the Colorado River will aid water managers with protecting public health and the designated beneficial uses of the Colorado River.

The Colorado River begins in the Southern Rocky Mountains of Colorado and ends in northwestern Mexico. Lake Havasu is a reservoir on the Colorado River, formed by Parker Dam, which was constructed by the Bureau of Reclamation between 1934-1938. The reach of the Colorado River that was monitored for this assessment flows south from Lake Havasu to the Bill Williams Wildlife Refuge. The California/Arizona border essentially runs along the middle of the river for the entire reach.

A total of thirty water grab samples were collected from thirty targeted sampling sites in September and October, which included open water and near shore sites (See map, figure 1). Site selection targeted areas of frequent public use and accessibility, as well as historical occurrences of cyanobacteria. Several of the near shore sites had to be collected by watercraft due to limited terrestrial access to monitoring sites. Samples were collected in accordance with the Surface Water Ambient Monitoring Program's (SWAMP) Standard Operating Procedures (SOP). Collected samples were shipped to Bend Genetics Laboratories located in Sacramento, California, and analyzed using Enzyme Linked Immunosorbent Assays (ELISA).

Figure 1: Sampling sites along the Colorado River, from Lake Havasu to the Bill Williams Wildlife Refuge

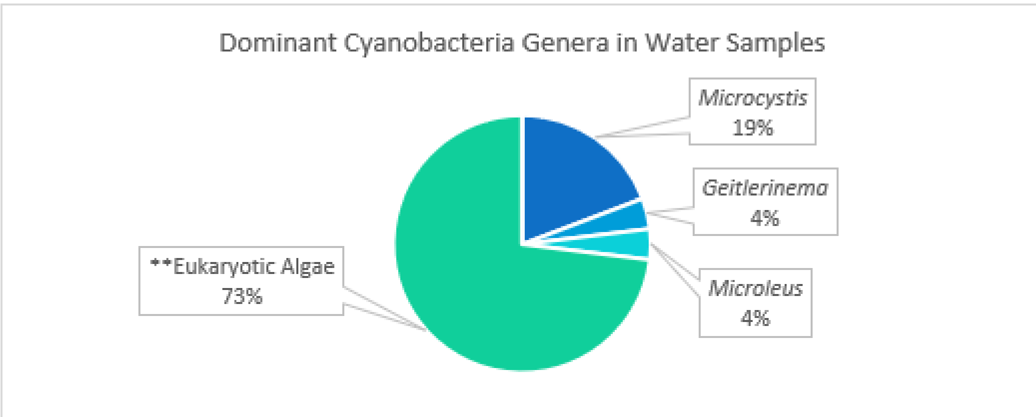
Table 1: Identification and GPS coordinates of sampling sites

Map ID		Site Name	Tentative Site Codes	Lat	Long
001	AZ	Castle Rock	714CSROCK	34.564134	-114.393816
002	AZ	Cattail Cove	714CATAIL	34.355357	-114.173578
003	AZ	Balance Rock	714BALNCE	34.425436	-114.291359
004	AZ	Steamboat Cove	714STEAMB	34.399443	-114.257218
005	AZ	Havasu State Park North Launch Ramp	714NORAMP	34.491278	-114.361193
006	AZ	Havasu State Park PWC Launch Ramp	714PWCRMP	34.494431	-114.361849
007	AZ	Kenny's Beach	714KENBCH	34.471732	-114.34874
008	AZ	Body Beach	714BODYBE	34.461392	-114.348489
009	CA	Skiers Island	714SKIERS	34.428813	-114.319379
010	CA	Copper Canyon	714COPPER	34.425016	-114.307624
011	AZ	B Williams River Refuge bridge	714BWRBRG	34.300537	-114.093712
012	AZ	Havasu Springs Resort	714HAVSPR	34.294101	-114.12054
013	CA	Whitsett Intake	714WHTSET	34.317356	-114.152129
014	AZ	London Bridge Beach	714LONBRG	34.465109	-114.34874
015	AZ	Hwy-95 cove	714H95COV	34.293649	-114.102366
016	AZ	B Williams River Refuge east of jetty	714BWRJTW	34.2929	-114.107971
018	CA	Open water 1	714OPENW1	34.364108	-114.221066
019	AZ	Open water 2	714OPENW2	34.303766	-114.12333
020	AZ	Parker Dam Launch Ramp	714PDRAMP	34.300778	-114.136769
021	CA	Open water 3	714OPENW3	34.38245	-114.242496
022	CA	Open water 4	714OPENW4	34.334903	-114.170306
023	CA	Whipple Bay	714WHIPLE	34.395903	-114.270002
024	CA	Black Meadow Landing	714BLKMDW	34.353267	-114.192135
025	CA	Open water 5	714OPENW5	34.406706	-114.288983
026	CA	Open water 6	714OPENW6	34.437322	-114.3295
027	AZ	Mile south of satellite cove	714SATCOV	34.327976	-114.146875
028	AZ	Half mile N. of Pistachio Cove	714PSTCHO	34.365677	-114.198821
029	CA	Vickie's Beach Cove	714VICKES	34.362031	-114.228861
030	AZ	BLM Facility Dock	714BLMDOC	34.441956	-114.31622
031	CA	Cove Near Gene Wash	714GENCOV	34.304892	-114.154776

Cyanobacteria Occurrence

Microcystis spp. was the most frequently detected genera for this assessment, being present in five of the thirty-one samples, four of which contained the highest concentrations of microcystin toxin. This would suggest that *Microcystis* spp. was the primary toxin producer, however, there were a total of fourteen samples where microcystin and cylindrospermopsin were detected in the absence of cyanobacteria detections, three of which exceeded the caution or warning level threshold. Only eukaryotic algae of unknown genera were observed in those fourteen samples. Given the brevity of duration for this initial assessment, these results may not be representative of temporal changes in cyanobacteria composition.

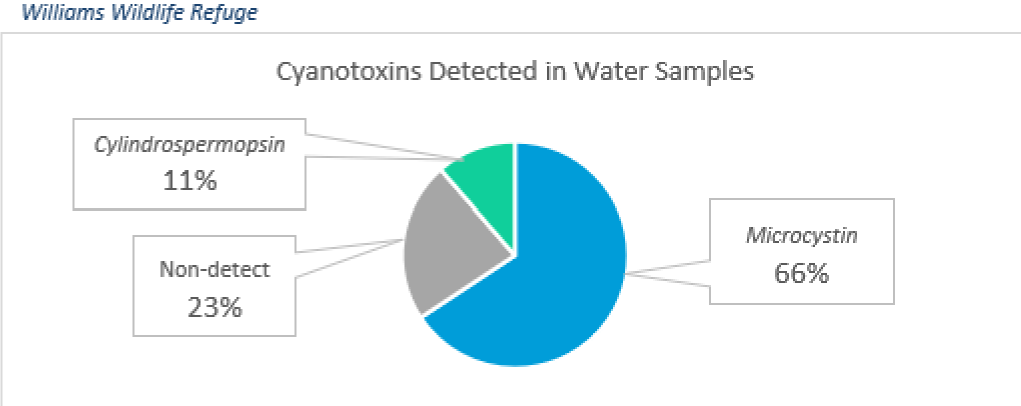
Figure 2: Frequency of cyanobacteria genera detected in water samples.



Cyanotoxins

Cyanotoxins are a concern for public health given the potency of their toxicity. The Office of Environmental Health Hazard Assessment (OEHHA) has established a set of trigger levels for detected cyanobacteria concentrations to be protective of human and animal health for planktonic Harmful Algal Blooms (HABs) in surface waters (Table 2), which have been adopted by the State Water Resources Control Board (SWRCB). When these trigger levels are exceeded, signage postings at the waterbody are recommended to alert the public of the dangers of planktonic HABs (Appendix B).

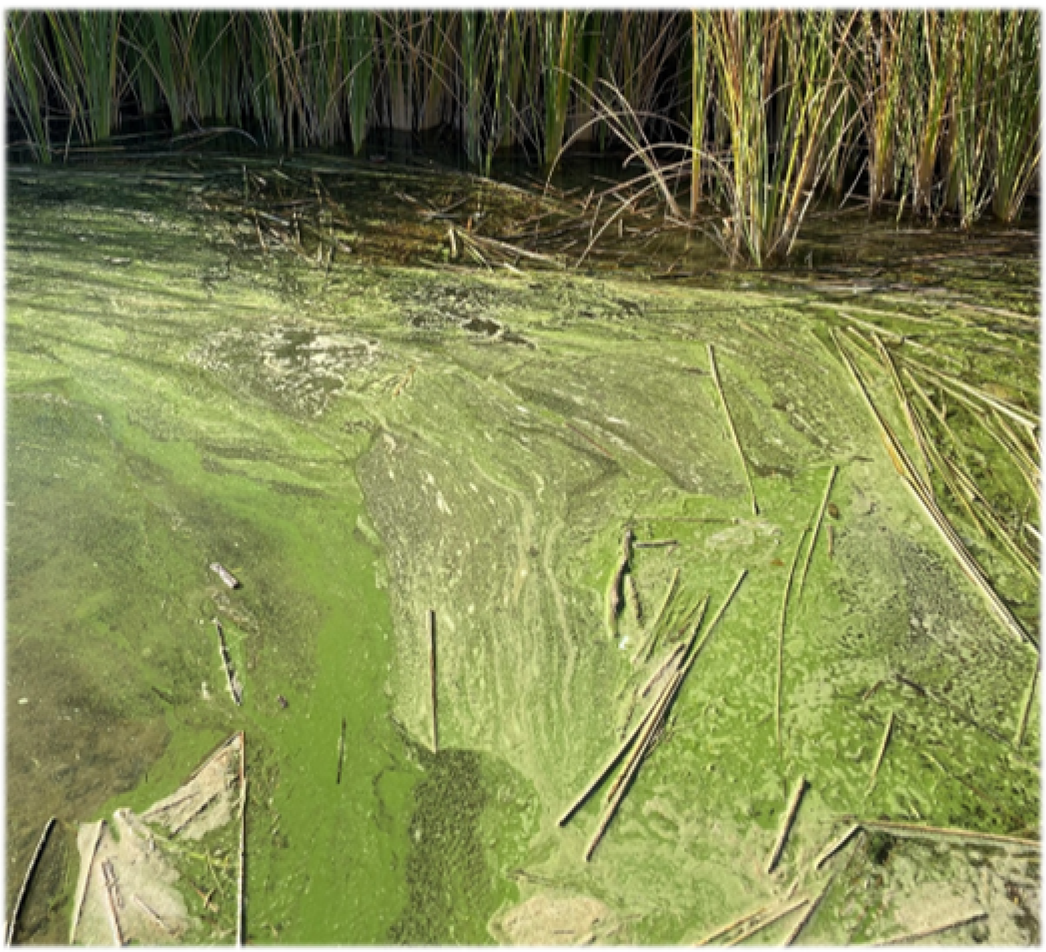
Figure 3: Frequency of cyanotoxin detections in samples collected between Lake Havasu and the Bill Williams Wildlife Refuge



All thirty water samples collected for this assessment were subjected to ELISA testing for quantitative analysis of anatoxin-a, saxitoxin, cylindrospermopsin and microcystins. Cyanotoxins were detected in 80% of the samples (24 out of 30), specifically microcystin and cylindrospermopsin, resp. Anatoxin-a and saxitoxin were not detected in any of the samples.

The dominant cyanotoxin observed in the samples was microcystin, a hepatoxin that primarily targets the liver. Typical symptoms of exposure to microcystin include diarrhea, vomiting, weakness, liver inflammation, liver hemorrhage, pneumonia, and dermatitis.

Figure 4: Harmful algal bloom near the personal watercraft launch ramp at Lake Havasu State Park. Microcystin concentration at this site measured 2,606 µg/L (Nov. 2022).



HABs Response

The State Water Resources Control Board (SWRCB) HABs response is based on the detection of cyanotoxins in surface water and algal mats, specifically anatoxin-a, saxitoxin, cylindrospermopsin and microcystin (Currently, the SWRCB does not have established thresholds for saxitoxin in water). Action levels developed by the Office of Environmental Health Hazard Assessment (OEHHA) have been adopted by SWRCB for the protection of public health. These response protocols prescribe three tiers of public health warnings: caution, warning and danger (Table 2). Each tier is associated with a specific response procedure based on the level of perceived risk (appendix A). For example, the trigger level of 0.8 µg/L for microcystin prompts increased monitoring as well as the posting of caution signs urging people to avoid scum, while recommending that pets

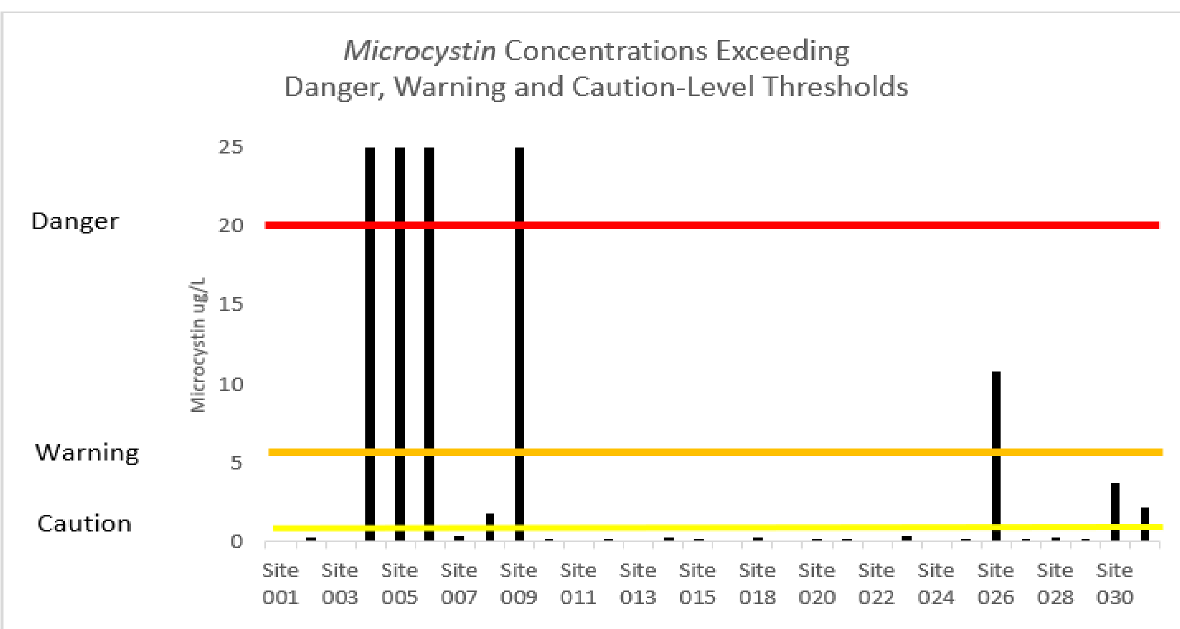
Table 2: Cyanotoxin thresholds for triggering the issuance of public health advisories

Trigger Levels For Human and Animal Health				
Criteria*	No Advisory [a]	Caution (TIER 1)	Warning (TIER 2)	Danger (TIER 3)
Total Microcystins [b]	< 0.8 µg/L	0.8 µg/L	6 µg/L	20 µg/L
Anatoxin-a	Non-detect [c]	Detected [c]	20 µg/L	90 µg/L
Cylindrospermopsin	< 1 µg/L	1 µg/L	4 µg/L	17 µg/L

and livestock be kept away from the water and scum. Alternatively, a danger warning recommends that people and animals stay away and off the water and avoid consuming any fish caught in that waterbody.

A total of eight samples from this assessment triggered the issuance of public health advisories whereby the Arizona State Parks Service promptly posted signage to inform the public, in accordance with the State Water Board's recommended protocols. Exceedances on the California side of the river were communicated to the San Bernardino County Public Health Department, but no advisory postings were placed around the waterbody for public safety. All data and information from this study was posted on the Water Board's managed public facing HAB web map. Of the eight samples triggering the advisories, four samples exceeded the *danger* level protocol threshold of 20 µg/L, one sample exceeded the *warning* level threshold of 6.0 µg/L, and three samples exceeded the *caution* level threshold of 0.8 µg/L.

Figure 5: Sampling Sites That Exceeded Danger, Warning and Caution-Level Thresholds for Microcystin



Water Quality Analytes

Temperature, specific conductivity, and dissolved oxygen were measured in-situ using a YSI® hand-held sonde (Pro DSS® model). Secchi disk readings for measuring water clarity were also recorded at each site. Additional parameters were analyzed by Bend Genetics laboratory including total nitrogen, total phosphorous, chlorophyl-a, and pheophytin-a (Table 3).

Table 3: In-situ and Other Water Quality Analytes Measured by Analytical Lab and Field Crew. Cells containing 'NA' indicate that data was not generated.

Map ID	Total-N (mg/L)	Total-P (mg/L)	Chlor-a (ug/L)	Pheo-a (ug/L)	DO (%)	Specific Cond. (µs/cm)	Temp (F)	Secchi Disk (feet)
001	1.54	0.04	9.26	9.18	NA	NA	NA	NA
002	0.83	<0.05	1.10	1.03	100.4	957	59.0	8.0
003	1.18	<0.05	0.82	1.22	96.7	948	58.1	7.5
004	3.09	0.30	169.72	75.72	96.4	948	58.4	7.5
005	1.60	0.05	120.38	70.49	NA	NA	NA	NA
006	62.80	0.50	2108.70	1189.90	NA	NA	NA	NA
007	0.80	<0.05	1.61	2.01	NA	NA	NA	NA
008	1.66	<0.05	1.87	2.83	NA	NA	NA	NA
009	3.65	0.23	196.35	231.31	97.3	955	57.8	8.0
010	0.66	<0.05	1.14	1.21	95.3	951	57.4	8.5
011	0.54	0.07	26.83	29.86	92.7	1113	53.5	1.1
012	0.67	<0.05	1.36	1.74	94.9	954	58.7	5.0
013	0.53	<0.05	1.20	1.51	95.6	954	58.9	7.0
014	1.53	<0.05	1.68	1.87	NA	NA	NA	NA
015	0.52	<0.05	1.31	1.66	95.3	951	59.3	5.5
016	1.10	0.02	7.36	12.13	NA	NA	NA	NA
018	0.76	<0.05	1.19	1.31	96.7	949	58.6	7.0
019	0.58	<0.05	1.18	1.53	95.3	951	59.0	6.0
020	1.58	<0.05	2.20	2.55	NA	NA	NA	NA
021	1.53	<0.05	1.01	1.22	96.8	951	58.1	6.5
022	0.83	<0.05	1.22	1.38	96.6	951	59.4	8.0
023	0.69	<0.05	1.33	1.49	97.7	938	58.0	8.0
024	0.81	<0.05	0.68	0.87	97.1	943	59.9	8.5
025	1.08	<0.05	0.81	1.14	96.5	938	57.4	6.0
026	1.19	0.03	20.87	14.75	96.4	948	57.2	6.5
027	0.62	<0.05	0.71	1.19	96.3	949	58.3	7.5
028	0.91	<0.05	0.63	0.76	102.9	954	58.7	9.5
029	0.66	<0.05	0.99	1.20	100.3	953	59.2	8.0
030	0.80	<0.05	15.94	9.61	100.9	954	58.6	>5.0
031	0.54	<0.05	3.03	2.64	91.1	969	56.7	>6.0

Table 4: Water quality parameters- high, low and median values

Analyte	Concentration				Units
	Low	High	Median	Mean	
Total Nitrogen	0.52	62.8	0.83	3.18	mg/L
Total Phosphorous	0	1.14	<0.05	0.08	mg/L
Spec. Conductivity	938	1113	951	958.1	ms/cm
Dissolved Oxygen	91.1	102.9	96.6	96.8	DO%
Chlorophyl-a	0.63	2108.7	1.35	90.08	µg/L
Pheophytin-a	0.76	1189.9	1.74	55.81	µg/L
Secchi disk	1.10	9.50	7.5	7.00	Ft.
Temperature	56.7	59.9	58.5	58.2	°F

Discussion

The findings of this assessment suggest that cyanobacteria is well established in the Colorado River from Lake Havasu to the Bill Williams Wildlife Refuge area. *Microcystis* spp. was dominant during this assessment, with *microcystin* being the most frequently detected cyanotoxin. It is important to note that the study occurred during the months of September and October of the same year. Therefore, the findings of this study may not be reflective of changing compositions of cyanobacteria during the summer growing season and/or toxin concentrations may vary during the heat of the summer months. The highest concentrations of chlorophyl-a and nitrogen were consistent with the highest *microcystin* concentrations detected in water. Phosphorous was only detected in one-third of all samples, with a single sample having >1.0 mg/L-P, suggesting that phosphorous may be a limiting factor for the proliferation of cyanobacteria in the study area. Potential sources of nutrients identified in the region thus far include septic systems, agricultural land use, golf courses and residential development.

The recreational sites monitored in this assessment are frequently used by the public, and full body contact occurs regularly. The observed distribution and intensity of cyanobacteria in Lake Havasu and the lower Colorado River during this assessment demonstrates a significant risk to the public, to wildlife, and to the local economy given the importance of Lake Havasu's tourism industry. These findings of elevated cyanotoxin concentrations triggered public health warnings for nearly one out of every three sampling sites. If Colorado River HABs follow current national trends of increasing frequency and magnitude, cyanobacteria blooms in the lake could worsen.

This assessment was the first time that the Colorado River Basin Regional Water Quality Control Board sampled HABs in Lake Havasu and the lower Colorado River, and so no other HABs data is available. The Regional Water Board has since discontinued its sampling for HABs in the Colorado River, however, a comprehensive monitoring program is strongly recommended for evaluating any spatial or temporal trends of cyanobacteria in the river that may indicate a worsening condition. Such monitoring would benefit from a collaborative effort incorporating concurrent assessment of nutrient loading and source tracking into the monitoring. Additionally, the exploration of HABs mitigation options would greatly benefit any effort to halt the proliferation of cyanobacteria in the river.

APPENDIX A

Appendix A. Description of cyanotoxin triggers in recreational waters.

This appendix describes the basis for the concentration levels selected to trigger the actions in the decision tree. The voluntary guidance relies on the science presented in OEHHA's risk assessment for microcystin, anatoxin-a and cylindrospermopsin (OEHHA 2012). Risk management decisions were used to integrate and expand the OEHHA action levels into a tiered response framework. Under this framework, increasing concentrations of cyanotoxins in recreational waters will prompt increasing public health warnings to users of the waterbody. Some of the triggers are not based on OEHHA's risk assessment but consider other important information such as animal poisoning reports and successful approaches used in other areas.

Development of this framework was a collaborative effort within CCHAB. Risk management decisions involve balancing the risk of low-level toxin exposures with the risks of closing waterbodies to the public, including economic, social and health impacts. Policy issues are also considered in risk management. The approach described here is designed to be feasible, useful and protective of public health.

Table A.1. CyanoHAB Triggers for Recreational Water.

	Toxin (µg/L)		
	Caution Trigger Level	Warning Tier I	Danger Tier II
Microcystins[1]	0.8	6	20
Anatoxin-a	Detect[2]	20	90
Cylindrospermopsin	1	4	17

[1]Microcystins refers to the sum of all measured microcystin variants.

[2]Must use an analytical method that detects <1 µg/L anatoxin-a.

Microcystin

The trigger level of 0.8 µg/L microcystin prompts increased monitoring and the placement of a caution sign stating that people should stay away from scum and pets and livestock should be kept away from the water and scum. The trigger level is based on the Office of Environmental Health Hazard Assessment's (OEHHA) action level of
0.8 µg/L (OEHHA 2012). The action level represents a concentration in recreational water that is not

expected to lead to adverse health effects. This is based on the best available science and very health-protective assumptions. OEHHA's action level is based on the short-term Heinze 1999 study in rats, which reported a Lowest Observable Adverse Effect Level (LOAEL) of 50 µg/kg-d. The endpoint was moderate liver pathology. The next highest dose (150 µg/kg-d) showed moderate to severe liver pathology. OEHHA calculated a 95% lower confidence limit of the Benchmark Dose (BMDL) of 6.4 µg/kg-d to represent the dose of microcystin that serves as the point of departure to estimate a safe dose for humans. An Uncertainty Factor (UF) of 1000 was applied to the BMDL, resulting in a Reference Dose (RfD) of 6×10^{-3} µg/kg-d, which estimates the dose of microcystin that is not expected to produce any adverse liver effects in humans. The UF of 1000 is the product of the following individual uncertainty factors: 10 for extrapolating from rat to human (assumes that humans could be 10 times more sensitive to microcystin than rats), 10 for the different sensitivities in humans (assumes the most sensitive person could be 10 times more susceptible to microcystin than the average person) and 10 for an incomplete toxicological database. The exposure scenario is a child swimming in recreational waters for 5 hours per day (30.25 kg child ingesting 0.05 L water per hour, or 0.25 L per day). OEHHA's risk assessment reflects a conservative, health-based approach and is described in detail in OEHHA (2012).

Managers should be aware of the risk to dogs and livestock when the microcystin trigger level is exceeded. Animals have been poisoned by microcystin at recreational waters in California. Exposure in dogs and livestock is unpredictable because they may consume scum at the shoreline and drying algal mats that wash up on shore. They are also exposed by cleaning cyanotoxin-containing material from their coats after being in the water. These materials may have much higher concentrations of microcystin compared to water levels. Dogs and livestock should be kept out of the water and away from the shoreline when the microcystin trigger level is exceeded.

The Tier I level of 6 µg/L microcystin in recreational waters would prompt the placement of a warning sign stating that swimming is not recommended and that pets and livestock should be kept away from the water. The Tier I level is based on OEHHA's calculated BMDL from the Heinze (1999) study (6.4 µg/kg-d; OEHHA 2012), a UF of 300 and a lower exposure rate. The cumulative UF of 300 includes the following factors: 10 for extrapolating from rodent data to humans, 10 for the differing sensitivities within the human population and 3 for an incomplete toxicological database. Using a UF of 3 for an incomplete toxicological database is a common approach in human health risk assessment and is often used by OEHHA (OEHHA 2008). Applying the cumulative UF of 300 to the BMDL of 6.4 µg/kg-d results in an RfD of 2×10^{-2} µg/kg-d. The exposure scenario is a child swimming in recreational waters for 2 hours per day (30.25 kg child ingesting 0.05 L water per hour, or 0.1 L per day). The Tier I level represents a health- based and conservative approach.

The OEHHA action level is conservative and its use as a posting trigger could lead to constant public warnings on waterbodies, which could obscure the need for caution when toxin concentrations increase. Policy and economic issues are also considered in risk management. Local authorities must weigh very low risks of toxic exposures with the high risks of closing waterbodies to the public, including economic, social and health impacts. The approach described here provides a highly useful tiered response to public health protection from microcystin exposure in recreational waters. The Tier I trigger is in the general range of OEHHA's microcystin subchronic action levels for water intake by dogs and livestock (OEHHA 2012). However, because these animals often consume scum and mats containing concentrated toxins, the action trigger level should be used for the protection of dogs and livestock from microcystin poisoning.

The Tier II level of 20 µg/L microcystin would prompt the placement of a sign stating that there is a present danger and that people, pets and livestock should stay out of the water and away from water spray. This level is based on risk management objectives rather than a purely health-based conservative approach. California water bodies support some of the highest microcystin levels in the world, with microcystins measured in the tens of thousands µg/L during bloom seasons in some areas. This guidance provides the Tier II "danger" level to convey the higher risk of critical liver impacts associated with higher microcystin levels in California waters. The concentration of 20 µg/L microcystin has been suggested as a warning level by the World Health Organization (WHO; WHO 1999). WHO's warning level is based on an earlier subchronic mouse study by Fawell et al. (1994; 1999a). Although OEHHA (2012) and USEPA (2015) found the later study by Heinze (1999; described above) to be a stronger study for the basis of health advisories, the Fawell study was acknowledged as a good study by both agencies. The WHO's warning level of 20 µg/L has been employed internationally for over a decade. There have been limited reports of human illness associated with recreational exposure to cyanobacterial bloom waters. Most of the reported human cases have involved rashes and gastrointestinal symptoms (Backer et al., 2015). The California Tier II level of 20 µg/L is intended as a severe warning level and is prompted by the historical, intermittent occurrences of very high microcystin levels in California waters.

Table A.2. Basis of suggested triggers for microcystin in Table A.1.

Basis of Trigger	Trigger (µg/L)	POD (µg/kg-d)	Total UF	RfD (µg/kg-d)	IR (L/d)	BW (kg)	Study
OEHHA's Action Level	0.8	6.4[b]	1000	6×10^{-3}	0.25	30.25	Heinze 1999
Modified OEHHA Action Level[a]	6	6.4[b]	300[c]	2×10^{-2}	0.1	30.25	Heinze 1999
Risk Management: *WHO Warning Level*	20	--	--	--	--	--	--

[a] OEHHA's BMDL with UF = 300 and lower exposure.

[b] Calculated BMDL from Heinze (1999) data.

[c] The UF is lower because a factor of 3 was used to account for limited database.

POD = Point of Departure, the study dose representing the lower end of the observed range of adverse effects.

IR = Ingestion Rate, how much water is assumed to be ingested by members of the target population BW = Body Weight, average for the target population being considered

Anatoxin-a

The trigger level for anatoxin-a is identified as any detection of the toxin in recreational waters. The chemical analysis must be able to detect anatoxin-a at levels below 1 µg/L in order for the trigger level to be useful. Concentrations above the trigger level will prompt increased monitoring and the placement of a caution sign stating that people should stay away from scum and pets and livestock should be kept away from the water and scum. The guidance for the trigger level is based on the precautionary approach in risk management. With this approach, known vulnerabilities may be addressed despite the absence of sufficient scientific evidence of risk. Anatoxin-a is a potent and very fast- acting neurotoxin. The toxin is responsible for numerous domestic animal and wildlife deaths. The trigger level is a precautionary measure, intended to prompt local managers to continue monitoring anatoxin-a concentrations to detect any increase. The anatoxin-a trigger level is also intended to protect dogs and livestock from acute poisoning as described below.

There is a heightened danger to dogs and livestock whenever anatoxin-a is present in a waterbody. Dogs and livestock may be particularly susceptible to acute lethality from anatoxin-a. Animal poisonings have been observed when anatoxin-a was present at low levels in California waterbodies. Animals can be exposed to high anatoxin-a concentrations by consuming scum at the shoreline and drying algal mats that wash up on shore. They are also exposed by cleaning cyanotoxin-containing material from their coats after being in the water. These materials may have high anatoxin-a concentrations even though the toxin levels are low in the water. Dogs and livestock should be kept out of the water and away from the shoreline when anatoxin-a is present.

The Tier I level of 20 µg/L anatoxin-a in recreational waters would prompt the placement of a warning sign stating that swimming is not recommended and that pets and livestock should be kept away from the water. The Tier I level is based on Oregon's recreational guideline for anatoxin-a, which incorporates a conservative approach (Farrer et al., 2015). The Oregon Health Authority (OHA) based their guideline on the short-term oral study in mice by Fawell et al., (1999b). The mice were examined for a wide range of toxicological endpoints both during and at the end of the study. There was no statistically significantly difference between the control group and any of the dosed groups for any of these endpoints. However there were two unexplained deaths in the study -- one each in the mid- and high-dose groups. Anatoxin-a was not suspected in these deaths but it was not possible to rule it out. Therefore OHA set the lowest dose, 100 µg/kg-d, as the No-Observed Adverse Effect level (NOAEL; Farrer et al., 2015). A UF of 1000 was applied to the NOAEL, resulting in an RfD of 0.1 µg/kg-d. The UF of 1000 is the product of the following individual uncertainty factors: 10 for extrapolating from rodent to human, 10 for the differing sensitivities within the human population and 10 for an incomplete toxicological database. The exposure scenario was a child swimming in recreational waters for 2 hours per day (20 kg child ingesting 0.05 L water per hour, or 0.1 L per day). OHA's risk assessment reflects a conservative, health- based approach using the best available science.

The OHA guideline was chosen as the Tier I trigger as a precautionary approach in the risk management of anatoxin-a. This concentration level is considered precautionary because it is more restrictive than the health-based OEHHA Action Level of 90 µg/L, described below. The Tier I level may be updated as more information becomes available. **As noted above, dogs and livestock are susceptible to acute anatoxin-a poisoning at water concentrations that are below the Tier I level due to high exposures in animals.** The action trigger level should be used for the protection of dogs and livestock from anatoxin-a poisoning.

The Tier II level of 90 µg/L anatoxin-a would prompt the placement of a sign stating that there is a present danger and that people, pets and livestock should stay out of the water and away from water spray. The Tier II level is based on OEHHA's action level for human exposure to anatoxin-a in recreational waters (OEHHA 2012). OEHHA's action level was also based on the short-term oral study in mice by Fawell et al., (1999b). As described above, there was no statistically significant difference between the control group and any of the dosed groups for a wide array of endpoints. OEHHA did not consider the two unexplained mortalities described above to be treatment related. The mice in this study were exposed daily through oral gavage, which is a somewhat stressful technique that can lead to unintended mortalities. OEHHA identified the highest dose, 2,500 µg/kg-d, as the NOAEL (OEHHA 2012). A UF of 1000 was applied to the NOAEL, resulting in an RfD of 2.5 µg/kg-d. The UF of 1000 is the product of the following individual uncertainty factors: 10 for extrapolating from rodent to human, 10 for the differing sensitivities within the human population and 10 for an incomplete toxicological database. The exposure scenario included a child swimming in recreational waters for 5 hours per day (30.25 kg child ingesting 0.05 L water per hour, or 0.25 L per day). OEHHA also considered exposures through inhalation and skin contact (OEHHA 2012). OEHHA's risk assessment reflects a conservative, health- based approach using the best available science.

Table A.3. Basis of suggested triggers for anatoxin-a in Table A.1.

Basis of Trigger	Trigger (µg/L)	POD (µg/kg-d)	Total UF	RfD (µg/kg-d)	IR (L/d)	BW (kg)	Study
Risk Management: *Precautionary Approach*	Detect	--	--	--	--	--	--
Risk Management: *OHA's Guideline*	20	100	1000	0.1	0.1	20	Fawell et al., 1999b
OEHHA's Action Level[a]	90	2,500	1000	2.5	0.25	30.25	Fawell et al., 1999b

[a] OEHHA's action level for anatoxin-a is not solely based on oral exposure (i.e., ingestion rate; IR). The action level also includes dermal and inhalation exposures. See OEHHA 2012 for details on the incorporation of dermal and inhalation exposures into the 90 µg/L action level.

POD = Point of Departure, the study dose representing the lower end of the observed range of adverse effects.

IR = Ingestion Rate, how much water is assumed to be ingested by members of the target population BW = Body Weight, average for the target population being considered

Cylindrospermopsin

The trigger level of 1 µg/L cylindrospermopsin prompts increased monitoring and the placement of a caution sign stating that people should stay away from scum, and pets and livestock should be kept away from the water and scum. The trigger level is based on a precautionary approach to the risk management of cylindrospermopsin. This toxin has been linked to a widespread outbreak of critical illness in humans following oral exposure (Byth 1980; Griffiths and Saker 2003). The details of the exposure concentrations are unknown but the outbreak supports the use of caution in managing risk associated with cylindrospermopsin. Additionally, less toxicological information is available for this cyanotoxin compared to microcystin. The trigger level concentration (1 µg/L) is intended to alert water managers to the possibility of toxic bloom formation.

Managers should be aware of the risk to dogs and livestock when the cylindrospermopsin trigger level is exceeded. Exposure in animals is unpredictable because they often consume material that can be very high in cylindrospermopsin (scums, mats, cyanobacteria stuck on their coats). We are not aware of any animal poisonings associated with exposure to cylindrospermopsin in California recreational waters. However, as a precaution, dogs and livestock should be kept out of the water and away from the shoreline when the cylindrospermopsin trigger level is exceeded.

The Tier I level of 4 µg/L cylindrospermopsin in recreational waters would prompt the placement of a warning sign stating that swimming is not recommended and that pets and livestock should be kept away from the water. The Tier I level is based on OEHHA's action level for human exposure to cylindrospermopsin in recreational waters (OEHHA 2012). OEHHA's action level is based on the subchronic study in mice by Humpage and Falconer (2003). OEHHA calculated a BMDL of 33 µg/kg-d. The endpoint was increased kidney weight, indicating mild impaired kidney function. A UF of 1000 was applied to the BMDL, resulting in an RfD of 3.3×10^{-2} µg/kg-d. The UF of 1000 is the product of the following individual uncertainty factors: 10 for extrapolating from rat to human, 10 for the differing sensitivities within the human population and 10 for an incomplete toxicological database. The exposure scenario is a child swimming in recreational waters for 5 hours per day (30.25 kg child ingesting 0.05 L water per hour, or 0.25 L per day). OEHHA's risk assessment reflects a conservative, health-based approach. The Tier I level is below OEHHA's cylindrospermopsin subchronic action levels for water intake by dogs and livestock. However, because these animals often consume scum and mats containing concentrated toxins, the action trigger level should be used for the protection of dogs and livestock from cylindrospermopsin poisoning.

The Tier II level of 17 µg/L cylindrospermopsin would prompt the placement of a sign stating that there is a present danger and that people, pets and livestock should stay out of the water and away from water spray. The Tier II level is based on OEHHA's BMDL from the Humpage and Falconer (2003) study described above. However, a lower UF for limited toxicological database is used (6) than was used in the OEHHA action level
(10). The cumulative UF of 600 was applied to the BMDL (33 µg/kg-d; Humpage and Falconer 2003), resulting in an RfD of 5.5×10^{-2} µg/kg-d. The UF of 600 is the product of the following individual uncertainty factors: 10 for extrapolating from rodent data to humans, 10 for the differing sensitivities within the human population and 6 for an incomplete toxicological database. The exposure scenario is a child swimming in recreational waters for 2 hours per day (30.25 kg child ingesting 0.05 L water per hour, or 0.1 L per day). The Tier II level is health-based and conservative.

Note that a higher cumulative UF (600) was used to modify the OEHHA action level for cylindrospermopsin compared to that used to modify the OEHHA action level for microcystin (UF=300). This is because more data is available for microcystin compared to cylindrospermopsin. The UF specific to a limited database was 6 for the modified cylindrospermopsin level and 3 for the modified microcystin level.

Table A.4. Basis of suggested triggers for cylindrospermopsin in Table A.1.

Basis of Trigger	Trigger (µg/L)	POD (µg/kg-d)	Total UF	RfD (µg/kg-d)	IR (L/d)	BW (kg)	Study
Risk Management: *Precautionary Approach*	1	--	--	--	--	--	--
OEHHA's Action Level	4	33	1000	3.3E-02	0.25	30.25	Humpage and Falconer (2003)
Modified OEHHA Action Level[a]	17	33	600[b]	5.5E-02	0.1	30.25	Humpage and Falconer (2003)

[a] UF is lowered from 1000 to 600.

[b] The UF is lower because a factor of 6 was used to account for limited database.

POD = Point of Departure, the study dose representing the lower end of the observed range of adverse effects.

IR = Ingestion Rate, how much water is assumed to be ingested by members of the target population BW = Body Weight, average for the target population being considered

References

Backer, L., et al. (2015). "Cyanobacteria and Algae Blooms: Review of Health and Environmental Data from the Harmful Algal Bloom-Related Illness Surveillance System (HABISS) 2007–2011." Toxins (Basel) **7**(4): 1048.

Byth, S. (1980). Palm Island mystery disease. *Med J Aust* **2**, 40, 42.

Humpage, A. R. and I. R. Falconer (2003). "Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value." Environ Toxicol **18**(2): 94-103.

Farrer, D., et al. (2015). "Health-Based Cyanotoxin Guideline Values Allow for Cyanotoxin-Based Monitoring and Efficient Public Health Response to Cyanobacterial Blooms." Toxins (Basel) **7**(2): 457.

Fawell, J. K., et al. (1994). Toxins from blue-green algae: Toxicological assessment of microcystin-LR and a method for its determination in water. Medmenham, UK, Water Research Centre: 1-46.

Fawell, J. K., et al. (1999a). "The toxicity of cyanobacterial toxins in the mouse: I microcystin-LR." Hum Exp Toxicol **18**(3): 162-167.

Fawell, J. K., et al. (1999b). "The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a." Hum Exp Toxicol **18**(3): 168-173.

Griffiths, D. J. & Saker, M. L. (2003). The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin. *Environ Toxicol* **18**, 78-93.

Heinze, R. (1999). "Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water." Environ. Toxicol. Pharmacol. 14(1): 57-60.

OEHHA (2008). Air toxics hot spots risk assessment guidelines: technical support document for the derivation of noncancer reference exposure levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

OEHHA (2012). Toxicological Summary And Suggested Action Levels To Reduce Potential Adverse Health Effects Of Six Cyanotoxins, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

http://www.swrcb.ca.gov/water_issues/programs/peer_review/docs/calif_cyanotoxins/cyanotoxins053112.pdf

WHO (1999). Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. London and New York, Routledge.
http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/