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February 21, 2020

VIA EMAIL
& Kate Fickas kfickas@utah.gov

Erica Gaddis
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P.O. Box 144870
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Joseph Miner
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UTAH DEPARTMENT OF HEALTH
P.O. Box 141000
Salt Lake City, UT 84114-1000

Re: Updated Regulatory Guidance Regarding Harmful Algal Blooms

Dear Ms. Gaddis and Mr. Miner:

We write on behalf of the Wasatch Front Water Quality Council (the "Council"), a coalition of wastewater treatment plants that funds research to better understand the Great Salt Lake, Utah Lake and Jordan River ecosystems. The Council provides the following comments on the updated joint Recreational Health Advisory Guidance for Harmful Algal Blooms (HABs) (the "State Guidance"), issued on January 22, 2020 by the Utah Division of Water Quality ("DWQ") and Utah Department of Health ("DOH") (collectively, the "Divisions"). While the Council appreciates that the Divisions updated the State Guidance to ensure that the public health advisory trigger levels for microcystin and cylindrospermopsin reflect current EPA guidance, the Council disagrees with the Divisions' continued reliance on cyanobacterial cell density to trigger public health advisory decisions.

The State Guidance provides for tiered levels for public health advisory, using cyanobacteria cell density, microcystin concentration, cylindrospermopsin concentration, and anatoxin concentration levels to establish three tiers of advisory. With respect to cell counts, cyanobacterial cell density from 5,000 cells/mL to 100,000 cells/mL could result in a "Warning Advisory," and cell density greater than 10,000,000 cells/mL results in a "Danger Advisory"

warning. The Council reiterates its comments from its November 1, 2019 letter (attached as **Ex. 1**) and subsequent January 6, 2020 Memorandum (attached as **Ex. 2**) that use of cell density is contrary to EPA Guidance and not a reliable advisory trigger. The Council further requests that the cell density factor be eliminated from the State Guidance.

The EPA Guidance focuses exclusively on concentrations of toxins microcystin and cylindrospermopsin for both use attainment in setting water quality criteria and to guide recreational health advisories.[1] In its 2019 guidance, EPA declined to make recommendations for issuing public health advisories based on cell counts. Specifically, EPA concluded that, with respect to using cyanobacterial cell density to guide health advisories, "available data are insufficient to develop quantitative recreational values"[2] and that given the inconsistency in the epidemiological studies, additional research is needed. The State Guidance nonetheless prescribes quantitative recreational values, contrary to EPA's conclusion.

As demonstrated in the Council's January 6, 2020 memorandum submitted by Leland Myers, significantly fewer warnings would have been issued in Utah last year were the EPA Guidance applied to the same data. Continued use of cell density as a trigger for public health warnings will continue to exaggerate the actual public health problem and create negative impressions about Utah Lake and other water bodies water quality even when toxin concentrations are not exceeded.

The Council requests that the State Guidance be amended to focus exclusively on toxin concentrations to guide public health advisories for HABs, rather than relying on imprecise and unsupported cyanobacterial cell density range as a factor. This revision is necessary pursuant to Utah Code Ann. §19-5-105, which provides that state standards developed in administering a program under the federal Clean Water Act can be no more stringent than federal standards addressing the same circumstances unless the agencies make a written finding, after public comment and hearing, that the corresponding federal standard is not adequate to protect public health and the environment.

If the Divisions insist on continuing to use cell density as a trigger for public health advisories, the Council requests that the Divisions eliminate the 5,000 to 100,000 cells/mL range for the "Warning Advisory" and replace it with a *permissive* advisory when the cell density exceeds 100,000 cells/mL. In that instance, local health departments would be encouraged to take into account other contextual information – in addition to cell density – and *consider* issuing an advisory. To achieve this, the Council suggests including the following language, which is similar to that contained in Table 1 of the proposed State Guidance: "Above 100,000 cells/mL, take into account other contextual information and consider issuing an advisory." This would

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[1] *See* Environmental Protection Agency; *Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin* (May 2019) ("2019 EPA Guidance").

[2] 2019 EPA Guidance at 17.

allow for consideration of various factors, including toxin concentrations, before a local health department issues an advisory based on cell counts alone.

The Council appreciates the opportunity to submit comments. Please contact me or Council Executive Director Leland Myers if you would like to discuss this matter.

Very truly yours,

Ashley A. Peck
Partner

AAP:bwt
cc: Wasatch Front Water Quality Council
Scott Baird

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Exhibit 1

HOLLAND&HART

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November 1, 2019

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Re: Regulatory Guidance Regarding Harmful Algal Blooms

Dear Ms. Gaddis and Mr. Miner:

We write on behalf of the Wasatch Front Water Quality Council (the "Council"), a coalition of wastewater treatment plants that funds research to better understand the Great Salt Lake, Utah Lake and Jordan River ecosystems. The Council respectfully requests that the Utah Division of Water Quality ("DWQ") and Utah Department of Health ("DOH") update their joint Recreational Health Advisory Guidance for Harmful Algal Blooms (HABs)("State Guidance") to be consistent with the U.S. Environmental Protection Agency's ("EPA") final Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cyclindrospermopsin (June 6, 2019)("Federal Guidance").

The State Guidance currently provides for tiered levels for public health advisory, using cyanobacteria cell density, microcystin concentration, cylindrospermopsin concentration and anatoxin concentration levels to establish three tiers of advisory. Cyanobacterial cell density greater than 20,000 cells/mL but less than 10,000,000 cells/mL results in a Tier 2 "Warning," and density greater than 10,000,000 cells/mL results in a Tier 3 "Danger" warning. Alternatively, a Tier 2 "Warning" can also result from a microcystin concentration of greater than 4 µg/L, a cylindrospermopsin concentration of greater than 8 µg/L[1], or a mere detection of anatoxin. A Tier 3 "Danger" warning can result from a microcystin concentration greater than 2,000 µg/L,

[1] The State Guidance cites to a 2016 draft of the Federal Guidance for this value.

potentially also from a cylindrospermopsin concentration of 8 µg/L,[2] or a concentration of anatoxin of greater than 90 µg/L.

Conversely, the Federal Guidance focuses exclusively on concentrations of toxins microcystin and cylindrospermopsin for both use attainment in setting water quality criteria and to guide recreational health advisories. The Guidance concludes that, in a primary contact recreational exposure scenario, a microcystin concentration of 8 µg/L and a cylindrospermopsin concentration of 15 µg/L would be protective of human health. Notably, EPA concludes that with respect to using cyanobacterial cell density to guide health advisories, "available data are insufficient to develop quantitative recreational values" and that given the inconsistency in the epidemiological studies, additional research is needed.

Accordingly, the Council requests that the State Guidance be revised to be consistent with the Federal Guidance. Specifically, a microcystin concentration of 8 µg/L and a cylindrospermopsin concentration of 15 µg/L should be used as values for Tier 2 "Warning" rather than the lower values currently used. We further request that the Guidance be simplified to focus exclusively on these toxin concentrations to guide public health advisories for HABs, rather than relying on imprecise and unsupported cyanobacterial cell density range as a factor. This revision is necessary pursuant to Utah Code Ann. §19-5-105, which provides that state standards developed in administering a program under the federal Clean Water Act can be no more stringent than federal standards addressing the same circumstances unless the agencies make a written finding, after public comment and hearing, that the corresponding federal standard is not adequate to protect public health and the environment. We request that DWQ and DOH finalize these changes to the guidance no later than Spring 2020 to ensure they are applied during the Summer 2020 recreational season.

Please contact me or Council Executive Director Leland Myers if you would like to discuss this matter.

Very truly yours,

Ashley A. Peck
Partner

AAP:bwt
cc: Leland Myers
Wasatch Front Water Quality Council
13686020_v1

[2] The State Guidance acknowledges that data are sparse on where cylindrospermopsin advisory points should be.

Exhibit 2

WASATCH FRONT
WATER QUALITY
COUNCIL

PROTECTING WASATCH FRONT WATERS THROUGH COLLABORATIVE, APPLIED RESEARCH

January 6, 2020

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RE: HAB Guidance Comments

Dear Sirs,

On behalf of the Wasatch Front Water Quality Council, attached is a comment memo on the Utah Recreational Health Advisory Guidance: Harmful Algal Blooms. This memo is in keeping with verbal comments offered at the December 11, 2019. Should you wish to discuss items in the memo, please contact me.

L Myers

Leland Myers
Executive Director, WFWQC

Comments on Utah HAB Guidance

Leland Myers, P.E. on behalf of Wasatch Front Water Quality Council

The Wasatch Front Water Quality Council (the "Council") provides the following written comments on the State of Utah's Harmful Algal Bloom ("HAB") Guidance. Members of the Council attended the Health Advisory Panel meeting on December 11, 2019 and write to reiterate our comments in writing. Pursuant to requests made at the meeting, the Council is also attaching follow up documentation to support its comments. This memorandum supplements a previous letter sent by Holland & Hart on November 1, 2019 and recommends: (I) that the State refine its anatoxin-a reporting to only report values that meet or exceed the laboratory reporting limit, and (II) that the State make changes to the current public health advisory tiers to be consistent with current EPA guidance and the approaches of other Western states. As discussed below, Utah's current tiered warning system leads to many unnecessary health advisories due to the use of cell counts, extremely low Anatoxin-a level triggers, and flawed sampling and reporting methodology.

I. Anatoxin-a Reporting.

The Department of Water Quality ("DWQ") and the Department of Health ("DOH") should no longer report Anatoxin-a values below 0.30 ug/L (the current reporting limit). After reviewing the State's reports of Anatoxin-a values, the Council identified numerous values below 0.30 ug/L, including some as low as 0.10 ug/L. The use of Anatoxin-a concentrations below the laboratory reporting limit ("RL") is inappropriate. While the detection limit is assumed to be 0.10 ug/L as per the Abraxis literature, the State Health Lab has stated that it uses an RL of 0.30 ug/L. Data below the RL is flagged as being unreliable. Indeed, the State Health Lab has stated that it periodically detects Anatoxin-a in the laboratory blanks. As long as the blank is less than one-half the RL, the testing is considered acceptable. This means that if the State continues to report below the RL, the value being reported is, at times, the same as that found in the blank. As per 40 CFR Part 136 Appendix B, spikes and blanks should be used to calculate a laboratory detection limit and a reporting limit developed therefrom. It is important for data used by DWQ and DOH in issuing health advisories and making public policy decisions to be valid and not subject to criticism as being unreliable. For this reason, the Council recommends that DWQ and DOH begin reporting only values that meet the reporting limit of 0.30 ug/L. Reporting correctly will provide accurate data to the public and will result in fewer unnecessary HAB warnings being issued.

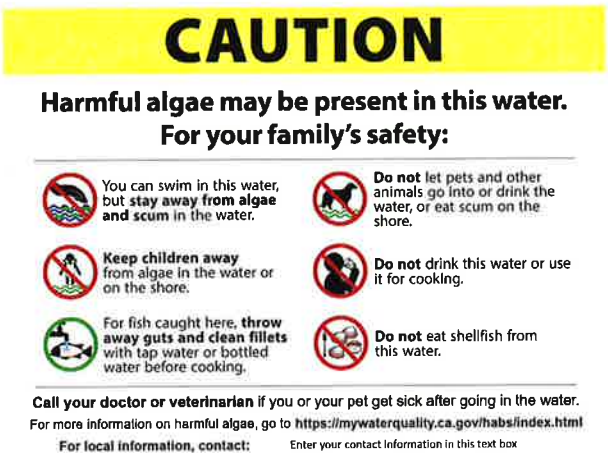
II. Recommended Changes to Public Health Advisory Tiers.

The Council recommends that the State make changes to the current DOH/DWQ HAB public health advisory tiers, as described below. This recommendation assumes that Anatoxin-a is included in addition to those given by EPA The recommendations in this section are consistent with 2019 EPA guidance and were modelled using the 2016 California Freshwater Harmful Algal Blooms Assessment and Support Strategy (Attachment 1) and the 2019 Oregon Harmful Algae Bloom Surveillance ("HABS") Program (Attachment 2). At the meeting on December 11, 2019 DOH/DWQ officials stated that they relied heavily on the California program when creating the Utah program. In addition, the Committee approved changing the trigger levels for Microcystins and Cylindrospermopsin to match the 2019 EPA guidance. However, the current State guidance does not reflect the 2019 EPA guidance. This memorandum relies on both the California program and the 2019 EPA guidance to make recommendations related to the following: (1) Use of Permanent Caution/Warning Signs; (2) Limited Use of Cell Counts; (3) Changing Warning Level for Anatoxin-a; and (4) Sampling Methodology. The recommended changes are depicted below:

LJM Proposal		
Tier 1	Tier 2	Tier 3
Low Risk	Moderate	High
	Whole Water Sample	Whole Water or Surface (Scum) Sample
Cyanobacteria Identified in Water Body	Microcystin >8	Microcystin >2,000
Permanent Caution Signs Installed at Access Points	Cylindrosperomopsin >15	Cylindrosperomopsin >15
Published on Internet	Anatoxin >10	Anatoxin >90
	Permanent Warning Signs Installed at Access Points	Permanent Warning Signs Installed at Access Points
	Published on Internet & Press Release	Published on Internet & Press Release
		Water Body Closed

1. Use of Permanent Caution/Warning Signs.

The State should begin using permanent caution or warning signs for waterbodies with HAB potential. One of the key reasons for establishing a HAB program is to inform the public of risks so they can make an informed decision on usage. When a water body is identified as having a bloom potential, it should have permanent caution/warning signs installed to alert the public of the possibility and provide basic information on risk minimization. California and Oregon take a similar approach. An example of a warning sign in California is shown below. The sign provides basic information and informs the water body user. It does not indicate that swimming should be avoided, but that water users should avoid identifiable algae or scum accumulation.



In Oregon, caution/warning signs caution the public "not [to] wade, swim or water ski in waters that have signs of an algae bloom." The Oregon guidance states that:

> "While waiting for laboratory analysis to determine if a recreational use public health advisory should be issued, local water body management may post educational and/or caution signs as a precautionary measure, to alert the public of potential health risks associated with recreating in a water body during a CyanoHAB.
>
> OHA has educational posters on the HAB webpage to use all year round, especially on waterbodies where blooms have been identified in the past."

An example of an educational sign from Oregon is shown below.

Again, Oregon does not warn people not to swim, but advises no contact with water when signs of algae are present. This signage also uses the tag line "When in doubt, say out!" This is clear and rememberable, thus providing a significant level of public health protection. This cautionary approach and the use of permanent signage informs the public of the potential risk and allows the user to make an informed decision. It would not create an over-reaction to the health risk. Recognizing that warning levels for HAB are based on a 1,000 factor of safety, the public would be adequately protected.

The Council recommends that DOH/DWQ implement a Tier 1 caution/warning level, which is consistent with the 2016 California trigger level for Caution as shown below:

Trigger Levels

Table 1 below provides recommendations to post advisory signs based on trigger levels for the following criteria: concentrations of three major cyanotoxins in water, cell count of potential toxin producers, and site-specific indicators. These trigger levels were developed to protect human and animal (dogs, livestock) health from cyanobacteria HABs; for more information on how these levels were derived refer to Appendix A - Description of cyanotoxin trigger levels in recreational water bodies. The advisory signs communicate potential risk to the public.

Table 1: Trigger Levels for Human and Animal Health

	Caution Action Trigger	Warning TIER I	Danger TIER II
Primary Triggers			
Total Microcystins[a]	0.8 µg/L	6 µg/L	20 µg/L
Anatoxin-a	Detection[c]	20 µg/L	90 µg/L
Cylindrospermopsin	1 µg/L	4 µg/L	17 µg/L
Secondary Triggers			
Cell Density *(Toxin Producers)*	4,000 cells/mL	--	--
Site Specific Indicators of CyanoHAB	Visible bloom/discoloration, scum, algal mats, satellite imagery,	--	--

[a] The primary triggers are met when ANY toxin exceeds criteria
[b] Microcystins refers to the sum of all measured microcystin congeners
[c] Must use an analytical method that detects ≤ 1 µg/L Anatoxin-a

The California Caution Action Trigger occurs when toxins are barely measurable and when cell counts are at a low level. As stated previously, Oregon similarly advises that all likely HAB locations have these permanent signs. As such, the recreational user values level in Oregon's guidance shown below tends to be similar to the Tier 1 warning level in the California Guidance shown above.

Table 2. Health advisory RUVs for cyanotoxins in Oregon recreational waters (µg/L)

*RUVs**	*Microcystin*	*Anatoxin-a*	*Saxitoxin*	*Cylindrospermopsin*
	8	15	8	15

*See Appendix B for the detailed rationale behind these RUVs.

Implementing this recommendation would make Utah's triggers for permanent signage consistent with California and Oregon. The next level—Utah's Tier 2 and California's Tier 1—would trigger the more serious signage as shown below.

Utah Warning Sign

California Warning Sign

This more serious signage should only be implemented when the Tier 2 toxin triggers recommended by the Council are met. If this recommendation were implemented, considerably fewer warnings would be issued. However, because of the permanent signage in HAB-prone water bodies, the public would still be effectively informed of the risks. As discussed below, Utah's current Tier 2 warning level leads to more advisories than are

necessary to protect public health. This is due to the use of cell counts, extremely low Anatoxin-a level triggers, and a flawed sampling methodology. Each of these will be discussed below, in turn.

2. Use of Cell Counts in the HAB Strategy.

The Council recommends that the State no longer rely on cell density as a factor for determining public health advisories. Instead, the State should focus on toxin concentrations, consistent with 2019 EPA guidance, to determine whether to implement advisories for HABs. The Council acknowledges that cell counts are a valuable tool in the identification of the potential for a HAB in a water body, and as such should be used for determining where permanent cautionary/warning signs should be installed. However, the presence of cells alone should not be used for issuing Tier 2 Warnings of potential health effects due to cyanobacteria presence. As noted in the May 2019 EPA "Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin," "[t]he EPA concluded that, although significant associations with adverse health effects occur across a wide range of cyanobacterial cell densities, the EPA cannot derive the CWA section 304(a) criteria based on total cyanobacterial cell density at this time." May 2019 EPA Recommendations at 96.

As shown in the guidance images above, neither California nor Oregon uses cell counts for their warning or advisory levels. Given the advancement and availability of toxin testing, regulators need not rely on the less accurate cell counts for warnings or closure. This is not to say that the use of cell counts should be discontinued entirely. They could continue as a means of identifying presence, potentially establishing Tier 1 Caution/Warning permanent sign installation and to assist in prioritizing sampling locations. The September 2019 EPA "Recommendations for Cyanobacteria and Cyanotoxin Monitoring in Recreational Waters" states, "Although EPA has recommendations for specific toxins, cell counts and/or biomass, together with microscopic identification can be informative and an interim step to make public health decisions and/or prompt toxin analysis." Sept. 2019 EPA Recommendations at 5.

Because the need for caution would be addressed with the permanent signs, movement to the Tier 2 Warning should be based solely on the 2019 EPA recommended toxin levels. While it is understood that cell presence may cause dermal irritation in a small percentage of the population, this alone does not justify moving to the Tier 2 Warning level. A research article in BMC Dermatology journal (Attachment 3) demonstrates the low level of impact cell counts alone have. The executive summary of that article states:

"This preliminary clinical study demonstrates that hypersensitivity reactions to cyanobacteria appear to be infrequent in both the general and dermatological outpatient populations. As cyanobacteria are widely distributed in aquatic environments, a better appreciation of risk factors, particularly with respect to allergic

predisposition, may help to refine health advice given to people engaging in recreational activities where nuisance cyanobacteria are a problem."

Therefore, as stated in the November 1, 2019 Holland & Hart letter:

> We further request that the Guidance be simplified to focus exclusively on these toxin concentrations to guide public health advisories for HABs, rather than relying on imprecise and unsupported cyanobacterial cell density range as a factor. This revision is necessary pursuant to Utah Code Ann. §19-5-105, which provides that state standards developed in administering a program under the federal Clean Water Act can be no more stringent than federal standards addressing the same circumstances unless the agencies make a written finding, after public comment and hearing, that the corresponding federal standard is not adequate to protect public health and the environment.

Using toxic concentrations, the Tier 2 advisory should only be implemented when levels are over 8 ug/L for Microcystin and over 15 ug/L for Cylindrosperomopsin. Again, these levels are consistent with 2019 EPA guidance, which the State has expressed a willingness to follow. As discussed below, the State should also change its Tier 2 value for advisories based on Anatoxin-a if it continues the use of Anatoxin-a at all.

3. Changing Warning Level for Anatoxin-a.

If the State insists on continuing to use Anatoxin-a despite it not being used by EPA, it should at minimum implement a more defensible value for Anatoxin-a for the Tier 2 warning level. As shown below, the Utah HAB guidance sets a current level for Tier 2 Warning as anything above detection of Anatoxin-a.

Table 1: UDOH/UDEQ HAB public health advisory tiers

	Tier 1: None	Tier 2: Warning	Tier 3: Danger
Relative Probability of Acute Health Risk [2]	Low	Moderate	High
Cyanobacterial Cell Density (cells/mL) [1]	< 20,000	20,000 - 10,000,000	> 10,000,000
Microcystins (µg/L) [1,3]	< 4	4 - 2,000	> 2,000
Cylindrospermopsin (µg/L) [3]	< 8	> 8 *	
Anatoxin-a (µg/L) [3]	Non-detect	Detection - 90	> 90
Additional Factors	None	Reports of animal illness or death	Reports of human illness
Health Risks [1]	Negligible	Potential for long-term illness Short-term effects (e.g., skin and eye irritation, nausea, vomiting, diarrhea)	Potential for acute poisoning Potential for long-term illness Short-term effects (e.g., skin and eye irritation, nausea, vomiting, diarrhea)
Recommended Actions	None	Issue WARNING advisory Post WARNING signs Sampling recommended at least weekly	Issue DANGER advisory Post DANGER signs Consider CLOSURE Sampling recommended at least weekly

At the December 11, 2019 meeting, the Health Advisory Panel claimed that this level was based on, "CSWB, 2016. California State Water Board: Cyanobacteria guidance for recreational and

related water uses (2016 updates)." However, the State's standards diverge from California's guidance, which is depicted in the table below:

Table 1: Trigger Levels for Human and Animal Health

	Caution Action Trigger	Warning TIER I	Danger TIER II
Primary Triggers			
Total Microcystins[2]	0.8 µg/L	6 µg/L	20 µg/L
Anatoxin-a	Detection[3]	20 µg/L	90 µg/L
Cylindrospermopsin	1 µg/L	4 µg/L	17 µg/L
Secondary Triggers			
Cell Density *(Toxin Producers)*	4,000 cells/mL	--	--
Site Specific Indicators of CyanoHAB	Visible bloom/discoloration, scum, algal mats, satellite imagery	--	--

[1] The primary triggers are met when ANY toxin exceeds criteria
[2] Microcystins refers to the sum of all measured microcystin congeners
[3] Must use an analytical method that detects ≤ 1 µg/L Anatoxin-a

As shown in the table, the California use of detection for Anatoxin-a is for the Caution Trigger, not for the Warning Trigger. If the State adopts the Council's recommendation to begin using permanent warning signs based on detection of Anatoxin-a, the State's guidance will align with that of California. It would then also be appropriate to use California's Tier I-Warning value for Anatoxin-a of 20 ug/L to inform Utah's trigger for Tier 2 advisories. This is consistent with the 2015 EPA "Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A." Oregon has used the toxic end points quoted in the EPA support document for their determination of a warning level for Anatoxin-a. The information below is extracted from the Oregon guidance contained in Attachment 2:

OHA used the TDI of 0.1 µg/kg-day above to derive a provisional **recreational use value of 15 µg/L for anatoxin-a:**

$$\text{Recreational Use Value} = \frac{\text{TDI} \times \text{RSC} \times \text{BW}}{\text{IR}}$$

Where:

TDI = Tolerable Daily Intake (0.1 µg/kg-day)
RSC = 1.0 (U.S. EPA 2000a; Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin 2019)
BW = Mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
IR = Recreational water incidental ingestion rate for children (0.21 L/d) at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997; Dufour et al. 2017)

In Utah, according to the DWQ HAB website, there were 107 samples that exceeded the detection limit current threshold in 2019. Of these 107 samples, the results of 99 were below the Utah Health Lab reporting limit of 0.3 ug/L and are therefore unreliable. Of the remaining 8 samples with concentrations that were actually detectable, only one was above 1 ug/L. If the recommendation set forth in this memorandum of 10 ug/L, the Oregon RUV level of 15 mg/L, or the California warning level of 20 ug/L were used there would have been no Anatoxin-a

warnings issued in 2019. This further demonstrates that the State should focus on the use of Microcystin and Cylindrosperomopsin, consistent with EPA Guidance.

The Council has conferred with a respected testing laboratory for cyanobacteria, and the laboratory confirmed when using reliable testing methodology, they very rarely see any significant concentration of Anatoxin-a in the samples they analyze. Based on this analysis, the Council respectfully requests that the State change its guidance to use a more defensible value of 10 ug/L for Anatoxin-a for the Tier 2 – Warning level, or ideally to eliminate the use of Anatoxin-a at all

4. Sampling Methodology.

In 2016, Utah developed a good sampling guidance document titled "Recommended Standard Procedures for Phytoplankton Collection to Detect Harmful Algal Blooms." This document defines two types of samples. Type 1 are surface samples and type 2 are composite samples. The Council agrees with both types of samples but disagrees about when they should be used, particularly for HAB Tier 2 Warning Levels. The State currently uses surface samples for all tiered warnings under the Guidance, which also has the potential to misrepresent the severity of the problem in a water body. Type 1 surface samples should only be used when widespread scum levels are identified, and testing determines the Tier 3 danger levels have been reached. Type 2 composite samples should be used to determine Tier 2 Warning levels. Many other states have adopted similar philosophies. For instance, Rhode Island adopted such a procedure that requires that:

> "Ankle-deep water samples should be collected approximately 15 cm below the water surface. Knee- and hip-deep water samples should be collected approximately 30 cm below the surface. The sample bottle should be inverted upon contact with the surface water to the appropriate depth and then scooped upward."

USGS sampling procedures also follow such guidelines, but they do still allow for scum sampling. Because ambient wind conditions and the buoyant capabilities of cyanobacteria dictate the presence or absence of scum layers, its intermittent nature means sampling events may or may not capture their potential presence. The permanent warning signs provide guidance to users to avoid such scum. Again, repeating the phrase "When in Doubt Stay Out" in signage provides a means of alerting the public that scums could occur any time. When toxin levels become high enough in the composite samples, the more stringent Tier 2 Warning will be issued.

The State should amend its current approach of using surface to only use composite samples for Tier 2 Warning Levels.

III. Conclusion.

The Council requests that the State amend its guidance to be consistent with the 2019 EPA guidance. The State should use permanent caution signs for waterbodies with HAB potential. The Tier 2 values for Microcystin and Cylindrosperomopsin should be amended to be 8 ug/L and 15 ug/L, respectively, and the mere detection of Anatoxin-a should not trigger Tier 2 warning levels. The setting of artificially low warning levels has the potential to create listener fatigue amongst the public, cause confusion as to when the public is truly at risk and even result in the public ignoring concerning levels. These adjustments to Utah guidance will provide warnings when needed and are justified in accordance with EPA guidance.

Attachment 1

2016 California Freshwater Harmful Algal Blooms Assessment and Support Strategy Strategic Plan - Phase 1

SWAMP
Surface Water Ambient Monitoring Program

Strategic Plan – Phase 1	2016

California Freshwater Harmful Algal Blooms Assessment and Support Strategy

Beverley Anderson-Abbs
Meredith Howard
Karen Taberski
Karen Worcester

SWAMP-SP-SB-2016-0001

January 2016

California Water Boards

www.waterboards.ca.gov/swamp

California Freshwater Harmful Algal Blooms Assessment and Support Strategy

California State Water Resources Control Board

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January 2016

Acknowledgements

The authors wish to thank the members of the Advisory Committees and other participants for assisting with the development of content for this document and reviewing draft versions. The Committee members are listed below in alphabetical order:

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I. Executive Summary

Harmful algal blooms (HABs) and algal toxins have increased globally in geographic range, frequency, duration, and severity in recent years. These increases have been attributed to various anthropogenic factors; the most significant include climate change, nutrient loading, and water residence time. HABs are problematic because they can affect multiple beneficial uses including recreation, aquatic life, and drinking water by reducing aesthetics, lowering dissolved oxygen concentration, causing taste and odor problems, and producing potent toxins. In recent years, cyanobacteria blooms and their associated toxins have gained national attention due to the severity of issues in the Midwest, and resulted in the release of health advisory values for drinking water by U.S. Environmental Protection Agency.

In California, toxic HABs caused by cyanobacteria (CyanoHABs) have been a recurring and escalating issue throughout the state, particularly in the Klamath River watershed, Clear Lake, Pinto Lake, Sacramento and San Joaquin River Delta, Lake Elsinore, and East San Francisco Bay Area lakes. Additionally, Copco and Iron Gate Reservoirs, the Klamath River, and Pinto Lake were placed on the State's 303d list due to impairment caused by cyanotoxins. In 2012, the State's Surface Water Ambient Monitoring Program (SWAMP) sponsored a statewide workshop in response to the growing concern about cyanotoxins. One of the key recommendations from the workshop was to develop a statewide long-term vision and strategic plan to address CyanoHABs and other freshwater HABs.

The goal of the California Freshwater HAB Assessment and Support Strategy is to articulate a coordinated and widely supported long-term program to assess, communicate, and manage CyanoHABs, cyanotoxins, and other nuisance freshwater HABs. The Assessment and Support Strategy framework has 3 components: (1) response to HAB events; (2) field assessment and ambient monitoring programs; and (3) risk assessment for potential HAB events. There are several components of infrastructure needed in order to support and implement this Strategy:

- *Satellite imagery* to identify and track cyanobacteria blooms
- *Centralized website and reporting system* to provide data management, visualization, and reporting capabilities
- *Guidance documents* on event response and management strategies
- *Laboratory resources* to support local event response
- *Training* on HAB characteristics and use of guidance documents
- *Applied research and tool development*
- *Outreach* aimed at providing educational materials to policymakers, health care professionals, veterinarians, and the public

SWAMP will provide funding towards the first five components of this infrastructure. USEPA has and will continue to provide significant laboratory resources for bloom response.

Satellite imagery will be used for (1) notifying waterbody managers of blooms detected in their waterbody, (2) a biweekly (during bloom season) bulletin and quarterly newsletter,

(3) an historical analysis of trends in large waterbodies, (4) reporting status of blooms on the website, and (5) informing status and trends monitoring.

A publicly available, centralized website is under development with the capacity for (1) database management and storage, (2) data access (downloadable), (3) data visualization, and (4) centralized information exchange for both reporting a bloom and notifying a wide audience of a bloom advisory or HAB event.

Guidance documents are an integral component of the infrastructure needed to support assessment and monitoring of HAB events. There will be three event response guidance documents to describe actions to employ during a HAB event, and provide a consistent set of procedures for water resource managers to follow including sampling, health and safety, and performance based quality assurance criteria.

The California Cyanobacteria Harmful Algal Bloom (CCHAB) Network, a multi-agency workgroup with representatives from state, local, and federal agencies, tribes, and the regulated, academic, and nonprofit communities, is developing waterbody posting and public notification, and toxin thresholds to update the 2010 Draft Voluntary Guidance. A summary of the updated Voluntary Guidance will be included in the event response guidance.

A comprehensive training program is being developed that focuses on all aspects of the guidance documents and is aimed at water resource managers, regulators, and agencies that conduct field sample collection and laboratory analysis.

Applied research is necessary to advance the technological development of methods related to satellite imagery analysis, toxin detection and analysis, automated optical identification of taxa, and mitigation and remediation.

The Outreach component, once funded, should be aimed at citizens, policymakers, health care providers and veterinarians, and public agencies (such as city municipalities, county health agencies). There is a critical need to increase public awareness of HABs including increasing recognition, public safety, and timely reporting of HABs, or associated events. There is also a need to develop a network of agency staff, waterbody managers, tribes and environmental health departments, and associated protocols for communication and coordination when cyanobacteria blooms occur.

Although SWAMP has funded much of the infrastructure of the Assessment and Support Strategy, there are still components that will need to be performed by other agencies and groups. HABs and associated toxins relate directly to the missions of a wide range of agencies in California; therefore identification of the mission and role of each agency, and coordination of these various agencies will lead to efficient use of all resources directed at HAB monitoring and mitigation throughout California. The purpose of this document is not to assign tasks, but to develop a framework for discussion by the involved agencies and the CCHAB Network so this strategy can be coordinated and implemented.

II. Introduction

A. Freshwater Harmful Algal Blooms in the Environment

Under certain environmental conditions in freshwater systems, single celled bacteria, called "cyanobacteria", can increase rapidly in biomass resulting in a "harmful algal bloom" (HAB), which in some cases can produce toxins. HABs can have negative impacts on the environment and raise serious concerns for drinking water sources, recreational use, pets, wildlife, and livestock. Additional information on harmful algae in the freshwater environment can be found in Appendix A. In recent years, harmful algae blooms from cyanobacteria (CyanoHABs), and associated cyanotoxins, have gained national attention due to increases in the frequency and severity of blooms, and their impacts on drinking water sources.

There are several well-documented problem areas in California that have been monitored through either assessment studies or water quality monitoring programs. Some of the areas with recurrent toxic cyanobacteria blooms include the Klamath River watershed (including Copco and Iron Gate Reservoirs), Clear Lake, Pinto Lake, lower Sacramento and San Joaquin Rivers and Delta, Lake Elsinore and several East San Francisco Bay Area lakes. The Klamath River and Pinto Lake have been placed on the State's 303d list due to impairment caused by cyanotoxins. More details on several of these programs are found in Appendix B.

More recently, cyanobacteria and cyanotoxin data have been collected opportunistically through several programs. These data indicate that cyanobacteria are prevalent throughout California in all types of waterbodies sampled (lakes, rivers, streams, wetlands, estuaries and coastal). Recent statewide assessment surveys of wadeable streams found that benthic cyanobacteria and related cyanotoxins are widely present, suggesting that these streams can be a significant cyanotoxin source to receiving waters (Fetscher et al., 2015). In statewide studies conducted from 2007 through 2013, samples were collected from more than 1,200 wadeable stream reaches. Analysis revealed a high occurrence of potentially toxic benthic cyanobacteria taxa, and detection of microcystins in one-third of reaches and 34% of stream kilometers. Detected toxins included lyngbyatoxin, saxitoxins, anatoxin-*a*, and microcystins (Fetscher et al., 2015). Additionally, the State Water Quality Control Board's Surface Water Ambient Monitoring Program (SWAMP) has measured cyanotoxins in sediment at the bottom of major watersheds in a majority of sampling sites.

B. Agency Responses

The California Cyanobacteria Harmful Algal Bloom (CCHAB) Network provides a forum for bringing together agencies, tribes, and organizations. The CCHAB Network was first established in 2006 as the Statewide Blue-Green Algae Work Group, in response to record-setting toxin producing blooms in Klamath River reservoirs. That Work Group, in collaboration with the State Water Resources Control Board (SWRCB), California Department of Public Health (CDPH), and Office of Environmental Health and Hazard Assessment (OEHHA), developed a draft guidance

document (*"Cyanobacteria in California Recreational Water Bodies: Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification"*) to provide background information on cyanoHABs, and establish thresholds for posting and lifting advisory warnings for bloom-affected waterbodies. In 2010, the group updated the guidance document and included a decision tree for posting health advisory warnings and recommendations for health advisory warning signs. The 2010 draft guidance document can be found at: http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/BGA/BGAdraftvoluntarystatewideguidance-07-09-2010.pdf.

In 2012, the working group was renamed and formalized into the CCHAB Network to coordinate efforts in addressing HABs throughout California. The CCHAB Network has representatives from federal, state, and local agencies, tribes, and the regulated, academic, and nonprofit communities. The CCHAB Network recently became a new Workgroup of the California Water Quality Monitoring Council (Monitoring Council) in order to strengthen ties between water quality programs, and is developing a cyanoHABs web portal for the Council's "My Water Quality" website (http://www.mywaterquality.ca.gov/). CCHAB is currently updating the 2010 draft guidance document.

In 2012, SWAMP sponsored a statewide workshop that included participants from regulatory, state, federal, and local agencies, scientists, resource managers, and non-governmental organizations, as well as several national cyanobacteria experts. The following key recommendations were developed at the workshop (this document fulfills recommendation #1):

1. Develop a long-term vision and strategic plan for statewide coordination to address cyanotoxins.
2. Develop and prioritize multi-agency management actions needed and identify the near-term policy actions of the various agencies responsible for freshwater HAB management.
3. Synthesize existing information and monitoring efforts and identify data gaps.
4. Develop standardized protocols for sampling and analytical methods.
5. Develop communication tools for sharing, accessing, and communicating HAB events and information.
6. Identify the best use of SWAMP monitoring and assessment resources, as well as additional partnerships and funding to support the long-term strategy.

In 2012, the Centers for Disease Control (CDC), U.S. Environmental Protection Agency (USEPA), and the U.S. Geological Survey (USGS) established the Inland Harmful Algal Blooms Discussion Group to continue and enhance communication on inland harmful algal bloom issues nationwide. This informal discussion group includes representatives from over 40 states, including California. An event that heightened awareness of the risks of cyanobacteria blooms occurred in 2014, when approximately 500,000 residents of Toledo, Ohio were without drinking water for several days as a result of a cyanobacteria bloom that impacted the city's source water, Lake Erie.

In 2015, the USEPA released health advisories for drinking water for the cyanobacteria toxins, microcystin and cylindrospermopsin. Health advisories provide states, drinking water utilities, and the public with information on health effects of microcystins and cylindrospermopsin, analytical methods to test for cyanotoxins in water samples, and treatment technologies to remove cyanobacteria toxins from drinking water. The USEPA included cyanobacteria and cyanotoxins on the drinking water Contaminant Candidate List as constituents that may require regulation under the Safe Drinking Water Act. Toxin thresholds for drinking water (USEPA, 2015) and recreational water (OEHHA, 2012) have been developed and are shown in Appendix C.

Currently, 22 states have freshwater HAB monitoring programs that vary significantly in purpose and organization, as well as type and frequency of monitoring (http://www2.epa.gov/nutrient-policy-data/states-freshwater-habs-monitoring-programs). SWAMP has initiated a Freshwater Harmful Algal Blooms (FHAB) program to help build the infrastructure for assessment, as well as provide support for dealing with freshwater HABs in the State of California.

C. Purpose of this Document

The purpose of this Strategy is to develop a program to inform management decisions for protecting public health and the environment. It provides a roadmap for the tools and guidance needed to support agencies and organizations as they address harmful algal blooms in freshwater. This document is intended to provide a framework for the Water Boards and other agencies to move forward in addressing freshwater HABs in a coordinated way.

Figure 1 shows the components of the Strategy. Some of the components are currently funded by SWAMP or other agencies, but there are other components for which resources and responsible agencies will need to be identified. The purpose of this document is not to assign tasks, but to develop a framework for implementation by SWAMP, the CCHAB Network and other concerned parties. It emphasizes infrastructure needed to manage blooms through monitoring, satellite imaging, information gathering and dissemination, remediation, and mitigation strategies (see Figure 2). In addition, funding mechanisms are identified that could potentially support this program in the long term. SWAMP has dedicated funding to this effort, but additional partners and resources will be necessary to fully implement this strategy.

The ideas in this strategy build upon elements already established through the SWAMP FHAB Program and the CCHAB, as well as through interviews with program leads from other states with established monitoring programs. The states contacted include Washington, Florida, New York, Vermont, Oregon, and Utah. In addition, members of CCHAB representing federal, state and local agencies, tribes, and scientific organizations within California provided input. It is necessary for these groups to participate in this strategy in order to achieve a coordinated response. A participants list can be found at the beginning of this document.

III. Freshwater HAB Assessment and Support Framework

The freshwater HAB assessment and support framework has three components, (A) response to HAB events, (B) field assessment and ambient monitoring, and (C) risk assessment for potential HAB events (Figure 1).

A. Response to HAB Events

The Response to HAB Events component focuses on the immediate monitoring and response actions applicable during a HAB event, including response to suspicious scum or illness, and mortality events potentially associated with HABs. It also includes the long-term actions needed to ensure an appropriate response to HAB events. Long-term actions include development of local action plans and implementation of management and remediation strategies. The infrastructure designed to support this strategy, especially in regard to event response, is described in detail in Section IV.

Event Response guidance documents are being compiled by SWAMP and CCHAB to provide expanded guidance on sampling, analysis, posting, thresholds, and remediation.

One of these documents, the CCHAB 2010 "Cyanobacteria in Recreational Water Bodies: Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification", will include waterbody posting and public notification guidance, as well as toxin thresholds for recreational exposure.

SWAMP is developing the second of these documents, a sampling and laboratory analysis guide that will include Standard Operating Procedures for field collection and laboratory methods, tiered approach to sampling and analysis, performance based quality assurance, and health and safety guidelines.

SWAMP is seeking funding for the final guidance document, a Management and Remediation Guide that will summarize available strategies and approaches for addressing a bloom. It should include summaries of the advantages and disadvantages of available methods, provide method selection criteria, give examples of success (or failure) for each method to mitigate or control HABs, and identify multi-objective benefits, considerations, and cost information associated with alternatives. Although SWAMP has funds for a workshop on this topic, which will be made available online, funding for a guidance document has not been identified.

These guidance documents will be available to the public and to all water quality regulatory agencies through the CCHAB Portal on "My Water Quality" (http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/) and the SWAMP website (http://www.waterboards.ca.gov/water_issues/programs/swamp/), and will be distributed to public health agencies, waterbody managers, drinking water source managers, water-based recreation companies, and wildlife organizations.

Another part of HAB event response includes the routine processing of satellite imagery for early detection of blooms in waterbodies. SWRCB (via SWAMP) will fund or provide this processing, for event response and for tracking trends. In spring 2015, the National Oceanic and Atmospheric Administration (NOAA) provided initial training for Geographic Information Systems (GIS) specialists in California to use the algorithms to process satellite imagery. This approach will provide immediate and cost-effective monitoring across a range of spatial and temporal scales not previously feasible using field-based monitoring, allowing for waterbody managers of waterbodies to be notified if a bloom is detected. Processed images will be posted online to a centralized website. Currently, San Francisco Estuary Institute (SFEI) has been contracted to perform these tasks.

SWAMP has limited laboratory resources that can be made available to waterbody managers for toxin sample analysis when other resources are not available. Criteria are being developed to guide the use of these resources, which will be coordinated with other analytical resources (e.g., USEPA resources for total microcystin analysis by enzyme-linked immunosorbent assay (ELISA) kits).

B. Field Assessments and Ambient Monitoring

The field assessments and ambient monitoring component is designed to assess the extent, status, and trends of HABs and associated toxins at the state, regional, watershed, or site specific waterbody scales.

Waterbodies or watersheds prone to HABs, or potentially at high risk based on risk assessments, should be routinely monitored in order to determine immediate health risks to the public, pets, wildlife, and livestock, and the impairment of other beneficial uses. Ideally, monitoring of these watersheds and waterbodies should include biological, chemical, and physical measurements to determine the following:

- Frequency, magnitude, and duration of HABs and toxins associated with a waterbody, watershed, or region
- Seasonality of blooms and toxin production
- Underlying drivers of HAB development

At a minimum, waterbodies that have experienced harmful algal blooms should be visually inspected frequently during the bloom season. If a bloom is suspected, water samples should be collected and analyzed for species composition. If a HAB species is identified, toxin analysis should be conducted.

HABs and associated toxins should be included as part of existing water quality, watershed, and volunteer monitoring programs where appropriate. Existing monitoring programs provide leveraged resources and additional information that can be used to identify and implement appropriate response, as well as methods of remediation or mitigation. HAB development is often tightly linked with sustained nutrient loading to waterbodies; therefore, nutrient

regulatory programs such as Total Daily Maximum Loads (TMDLs) should include HABs in their core analytes for data collection and analysis.

Citizen science and volunteer monitoring programs exist throughout California and can add HABs to their data collection efforts where appropriate. An example of one program that has been collecting cyanobacteria and toxin data is the Eel River Recovery Project, a citizen-based watershed monitoring and education group focused on the Eel River in Northern California (http://www.eelriverrecovery.org/) where anatoxin-a and cyanoHAB effects have been documented.

Environmental stressors associated with bloom initiation, maintenance, and toxin production need to be identified wherever possible. While these factors are being studied by many at the national and international levels, regional and local level information is critical to responding successfully to cyanoHABs. Understanding the drivers of bloom formation is critical to determining appropriate management strategies to successfully mitigate HABs, and to implement cost-effective, long-term remediation plans.

To understand the spatial extent of cyanobacteria and toxins in the environment, existing probabilistic field programs should be leveraged to include indicators associated with cyanobacteria blooms. These programs include the USEPA's National Aquatic Resource Surveys, SWAMP Bioaccumulation Monitoring Program, SWAMP Perennial Streams Assessment (PSA), and regional stormwater monitoring programs like the Southern California Stormwater Monitoring Coalition (SMC) and San Francisco Bay Area's Regional Monitoring Coalition (RMC).

Where possible, nutrients, chlorophyll-a, phycocyanin, cyanobacteria, and HAB algae identification and enumeration, as well as associated toxins, should be added to the list of analytes collected and analyzed by existing condition assessment programs. The inclusion of these indicators will provide improved datasets to characterize the extent of cyanobacteria blooms in the area assessed, assist in verifying satellite bloom reporting, and in identification of key environmental stressors associated with HAB development and persistence. The extent of waterbodies or watersheds affected by HABs and associated toxins can be estimated from such probabilistic assessments, and waterbodies or regions with recurring HABs can be prioritized for more intensive routine monitoring.

The limitations of adding cyanobacteria monitoring to existing efforts are: 1) most of these programs are in streams and rivers, and not in lakes where most of the recreational and drinking water use take place; and 2) this approach is inadequate for fully understanding the temporal or spatial variability of blooms. CyanoHABs can be very patchy; therefore waterbody-specific studies need more intensive study design, both in space and time, to understand these patterns. As funds become available, routine monitoring programs, specifically designed for freshwater HABs, will provide a mechanism to overcome the limitations of assessment based programs and to decipher spatial and temporal patterns in taxonomy, toxin production, and the stressors associated with blooms.

C. Risk Assessment

The main goal of risk assessment is to determine the target regions, watersheds, or waterbodies experiencing HABS, or at risk for HABs, to prioritize for assessment, monitoring, remediation, and risk management. The risk assessment approach can be used to assess large geographic areas with minimal resources as a first order mechanism to evaluate potential HAB issues in a region. This approach narrows the number of waterbodies for monitoring and assessment studies, since it is not fiscally feasible to monitor all waterbodies in a region, or throughout a large geographic area, like California.

Statewide Scale: There are three analyses that can be conducted at the statewide scale.

An ***historical analysis of blooms*** derived from satellite imagery, as well as other data, is being conducted, with SWAMP funding, to identify lakes larger than 100 hectares (~250 acres) that experience blooms, and to assess seasonal and spatial trends at individual waters and throughout the state. The historical analysis will address the period from 2002-2012, using imagery from the European Space Agency's (ESA) Medium-spectral Resolution Imaging Spectrometer (MERIS), and other data sources.

Ongoing satellite imagery analysis will identify waterbodies with recurrent harmful algal blooms. These waterbodies would be considered higher risk.

A ***landscape risk analysis*** could be conducted using GIS and remote sensing data to weigh risk factors and identify high-priority waterbodies for field assessments and monitoring. Existing GIS data layers such as waterbody types and beneficial uses, together with regional information about waterbody use by the public, pets, wildlife, and livestock, point and non-point nutrient sources, hydrological modifications, and current land use could be used to prioritize locations for monitoring in screening assessment. Additionally, collated records of historical blooms from ad hoc studies, remote sensing, or ambient monitoring could provide additional screening criteria.

The products from a landscape risk assessment could include a publically-available GIS interface that provides maps with layers addressing (1) waterbody locations, (2) beneficial use, (3) recreational water contact activities, (4) records of historical HABs, and (5) distribution and abundance of environmental stressors that may increase the probability of blooms. Each of these products could be developed as a separate assessment study. Together, the results of these studies could be used to prioritize waterbodies for monitoring and field assessments. However, though useful information may be derived from this effort, it is resource intensive and may not be predictive, and therefore is considered a lower priority than other components of this Strategy.

IV. Infrastructure to Support Monitoring and Assessment Strategy

There are several components of infrastructure needed to support and implement the Monitoring and Assessment Strategy, including (1) satellite monitoring for cyanoHABs, (2) a

centralized data management system with website and reporting capabilities, (3) guidance documents on a wide variety of topics, (4) laboratory resources, (5) training, and (6) outreach. Figure 2 summarizes these components.

A. Satellite Monitoring

The National Cyanobacteria Assessment Network (CyAN) Project is a collaboration of federal agencies including USEPA, NOAA, USGS, and National Aeronautics and Space Administration (NASA) working to integrate satellite information into water quality programs and management decisions nationwide. The CyAN project will expedite public health advisories through early detection of cyanoHABs. The satellite imagery used by the CyAN project provides a synoptic view of the development, and temporal and spatial distribution of cyanoHABs, by distinguishing between chlorophyll and phycocyanin (Wynne et al., 2010, Stumpf et al., 2012). Currently, the use of satellite imagery is limited to lakes larger than 100 ha (~250 acres), however research is being conducted to be able to detect blooms in smaller waterbodies using the Sentinel 2 and Landsat satellites.

California is one of several βeta-test states for the CyAN project. SWAMP has provided funding to support development of the methods for California and will be an early participant for receiving, processing, and posting satellite imagery online. SWAMP is developing a protocol to contact waterbody managers about when and where cyanobacteria blooms are occurring based on satellite information. SWAMP will support download, analysis, interpretation, and posting for web access to satellite imagery until this becomes available nationally via the CyAN Project (anticipated in 2020). After the CyAN project begins, this strategy assumes that much of the analysis will be conducted through the national program; however, California will continue to require download and interpretation of satellite imagery, and communication with water managers. This strategy assumes SWAMP will continue to provide this role.

B. Centralized Database and Website with a Reporting System

A publically available, centralized database and website is under development as part of the SWAMP Freshwater HABs program. This will have the capacity for (1) database management and storage of satellite and related data, with the website providing (2) data access (downloadable), (3) data visualization, and (4) centralized reporting capabilities for both public reporting of blooms or illness, and notification to a wide audience of a bloom advisory or HAB event. The website will be housed within the California Water Quality Monitoring Council's "My Water Quality" website. It is currently expected to include downloadable GIS data layers of bloom analyses, bloom maps, web-based data upload tools, incident reports (including animal and human illness or death), and bloom information. The reporting system will provide a mechanism for the public and water resource managers to report HABs, or suspected HAB events to help identify HAB hotspots. This system will facilitate water resource manager and agency awareness for sampling, posting, and closure of suspect waterbodies.

An associated database will store satellite information, as well as descriptive bloom information, and will be populated by data from waterbody managers and monitoring programs. A template will be provided that will enable upload of taxa and toxin data to the California Environmental Data Exchange Network (CEDEN). Data will be publically accessible and downloadable on the website, wherever feasible. The website will have mapping capabilities that allow for visualization of HAB datasets, and will be able to overlay other water quality datasets.

Existing websites, such as the one developed for the Klamath River, will be used as models for the statewide website. The Klamath Basin Water Quality Monitoring Program has a "blue-green algae tracker" interactive web map (http://kbmp.net/maps-data/blue-green-algae-tracker). The tracker uses current information to map cyanobacteria blooms throughout the Klamath Basin to inform the public, research, management, and stakeholder communities of the current conditions, bloom events, and health threshold exceedances. Other states have similar HAB trackers that can serve as models for the California website. Some of these report current HAB event locations and provide waterbody safety status, including information about alerts and advisories.

HAB data reporting will be conducted through the centralized website and will include the following:

- Reporting mechanism for the public, waterbody managers, veterinarians, and state and local agencies to report a bloom, or animal illness or mortality events potentially associated with a bloom
- Short-term, timely notifications of blooms and animal mortality events
  - These may be based on the blooms reported or satellite imagery
- Newsletter or bulletin issued on a routine basis providing information about blooms, toxins, and reports of illness or mortality
- Maps showing blooms based on reported events and satellite imagery of high biomass and cyanobacteria dominance.

HAB reporting applications are being developed for smartphones by USEPA as part of the CyAN project. These applications will be one way to meet California's reporting needs and should be capable of connection to the website to obtain access to HAB reports. Additionally, the application will access website data so the public can utilize smartphones to obtain the most current advisories and information. At this time, this is not part of the Freshwater HAB Program and would require additional resources to implement.

C. Event Response Guidance Documents

Guidance documents are an integral component of the infrastructure needed to support assessment and monitoring of HABs.

The ***Event Response Guidance documents*** will include:

- standard operating procedures for sample collection methods for multiple beneficial uses (including aquatic life, recreational contact, and drinking water) and waterbody types
- standard operating procedures for laboratory toxin and taxonomic analyses
- health and safety recommendations for laboratory and field sample handling
- performance based quality assurance guidance
- decision frameworks for sampling and analysis, and a protocol for waterbody manager response, and agency and public notification
- a summary of California's established toxin thresholds for protection of public and pet health in recreational waters including the CCHAB posting thresholds, as well as USEPA's health advisory values for drinking waters
- a list of agencies and laboratories to contact for sample analysis services and guidance, and for illness and mortality events
- remediation and mitigation guidance

SWAMP Freshwater HAB Event Response Guidance Plan - SWAMP is developing ***Standard Operating Procedures*** (SOPs) and quality assurance guidance documents for field sample collection in all waterbody types (lakes, rivers, creeks, wetlands, estuaries, and coastal waters), laboratory sample analysis methods, and health and safety recommendations. The main purpose of this guidance is to ensure that consistent sample collection and analysis are conducted throughout the state by field assessment and monitoring programs to facilitate comparisons of data across programs, and ensure the safety of all individuals.

The field collection SOP will include identification of indicators needed to determine the immediate risk to public health, wildlife, pets, and livestock. Detailed instructions on how to sample algae and cyanobacteria, and water column chemistry (including toxins) within different types of waterbodies will be provided in the SOPs. Sampling design considerations will be conveyed in the SOPs in order to ensure samples collected are representative and have sufficient statistical power. These methods will be designed to standardize data collection for all relevant parameters in order to meet quality assurance and quality control requirements, also detailed in these documents. The SOP will provide health and safety procedures to ensure the safety of all individuals involved in sample collection and handling. SOPs for most water quality parameters can be found on the SWAMP website:
http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#methods

The laboratory section will focus on sample preparation, analysis, and reporting and will include: (1) SOPs for all of the toxin and taxonomic identification methods, (2) performance based quality assurance parameters for each analysis method (e.g. sensitivity, percent recovery, reproducibility, unequivocal identification) for these methods, and (3) a ***decision framework*** for analyzing cyanobacteria and cyanotoxins for event-response sampling. The decision tree framework is important to determine the toxin(s) of interest, how the samples will be prepared,

the type of analysis that will be utilized, and how these data will be interpreted and used. The SOP will provide health and safety procedures to ensure the safety of all individuals involved in laboratory analysis.

At the time of this writing the SOPs discussed above are funded projects under the SWAMP Freshwater HABs Program, and are estimated to be completed in 2016.

As part of ***performance based quality assurance***, SWAMP has developed measurement quality objectives (MQOs) and sample handling requirements for cyanobacteria and cyanotoxin measurements for SWAMP funded monitoring projects and SWAMP comparable projects (http://www.waterboards.ca.gov/water_issues/programs/swamp/mqo_cyanotoxin.shtml). The intent of the MQOs is to ensure data collected for regulatory or waterbody assessment purposes are of known and consistent quality to support management decisions. Data collected for screening or research purposes will need to meet minimum requirements established by the method utilized or project specifications. The data generated by SWAMP and SWAMP comparable programs are made available to the public through CEDEN. The quality assurance objectives currently designed for SWAMP may be adapted for the purposes of other state water quality monitoring programs. Additional information can be found at the SWAMP Information Management and Quality Assurance Center (SWAMP IQ).

The recently released SWAMP MQOs focus on the two most common cyanotoxin analysis methodology groups used by SWAMP: ligand-binding assays (e.g. by enzyme linked immunosorbent assay or ELISA) and analytical chemistry assays (by liquid chromatography-mass spectrometry or LC-MS). SWAMP IQ plans to update MQO documents based on the needs of proposed SWAMP HAB monitoring.

The event response guidance will also include a ***list of agencies*** and laboratories to contact for sample analysis (including taxonomic identification and toxin analysis), and agencies that need to be notified when there is a bloom. The plan will be widely distributed to all water quality regulatory agencies, public health agencies, water resource managers, and wildlife rescue organizations.

CCHAB Guidance -"Cyanobacteria in California Recreational Water Bodies: Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification"
This document was developed in 2010 and is currently undergoing updates and revisions (http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/BGA/BGAdraftvoluntarystatewideguidance-07-09-2010.pdf).

The guidance includes, among other topics, the following components:

- decision tree for providing public notification, and posting and lifting advisory warnings for bloom affected waterbodies
- a narrative explaining the steps in the decision tree
- recommended types of sampling and frequencies
- cyanotoxin toxicity thresholds for recreational exposure

- description and basis of cyanotoxin thresholds
- examples of signage for public warning

The CCHAB Guidance will be an important component of the overall event response guidance for dealing with freshwater harmful algal blooms and associated toxins.

A ***Bloom Management and Remediation Guidance*** document is also needed, and should include watershed and waterbody approaches to HAB management. There are many methods currently utilized for management and remediation that include (but are not limited to) the following:

- Reduction of nutrient inputs
- Inhibition of internal nutrient loads from sediments (such as chemical treatments, floating treatment wetland technology or dredging)
- Mixing and destratification of the water column
- Increased flushing or flow rates to reduce water retention time
- Waterbody treatments with algaecides
- Biological manipulations
- Ultrasound

The guidance document should summarize the advantages and disadvantages of each of these methods and provide selection criteria and examples of success (or failure) of each method to mitigate or control HABs. Although SWAMP has funded training on management and remediation of cyanobacteria blooms, the guidance document is not currently funded.

D. Laboratory Resources

The SWAMP program has set aside some resources for ***laboratory analysis*** so that water managers with insufficient resources can identify blooms, and will be developing criteria for their use. USEPA also provides services for some waterbodies experiencing recurrent, serious blooms. However, additional resources for sample analysis need to be secured. Laboratories capable of conducting analyses will be listed in the Event Response Guidance Document. An ***inter-laboratory comparison*** should be conducted to ensure data is comparable across laboratories. Performance based quality assurance metrics should be documented for the inter-laboratory comparison.

E. Training

There are two types of training programs needed to ensure successful implementation of the Monitoring and Assessment Strategy. All of this training is currently funded through SWAMP.

A ***comprehensive training program*** focused on all aspects of the guidance documents, and geared toward waterbody and watershed managers, regulators, and agencies conducting field sample collection and laboratory analysis, is being developed. This training program will help improve awareness, recognition, and reporting of HAB events.

In-person training workshops are being conducted throughout the state. At the time of this writing, the SWAMP Freshwater HAB program is sponsoring multiple workshops to provide training on the following topics:

- HAB general information, including taxonomy and identification, and types of toxins
- Water quality and public health issues caused by HABs
- Programs and resources within CA
  - CCHAB voluntary guidance document
  - Toxin thresholds for recreational exposure
  - Management and mitigation strategies for HABs
  - SWAMP sampling and analysis guidance document
  - SWAMP Freshwater HAB program and CCHAB network

Training materials will be distributed to workshop participants and the workshops are being recorded and posted on YouTube, where they will be made available on the centralized website. SWAMP is conducting these trainings in summer 2015 and 2016.

A ***bloom management and remediation workshop*** is planned and will provide more detailed information on ways to address blooms. The material presented should be summarized in the "Bloom Management and Remediation" guidance document discussed above. This workshop will focus on tools for lake managers to address nutrient sources and mitigation techniques within lake environments.

F. Applied Research and Tool Development

Technology to support bloom identification and analysis is developing rapidly and California should continually evaluate and adapt programs, as appropriate, to take advantage of new analytical methods, imagery analysis approaches, and other tools. As such, research needs may arise to support adapting these technologies for our program needs. Not all of these can be anticipated, however, several currently developing methods are described below.

One immediate need is satellite imagery analysis to support bloom identification in smaller scaled waterbodies. The Medium Resolution Imaging Spectrometer (MERIS) satellite imagery currently being used to initiate the imagery analysis program is limited to larger waterbodies, as is the Ocean Land Color Instrument (OLCI) sensor on the Sentinel-3 satellite. Analysis from higher resolution imagery could be added to provide information on smaller waterbodies. Paired Sentinel-2 satellites with multi-spectral imaging will provide much higher resolution imagery. However, the algorithms to detect cyanoHABs need to be improved. This can be done by collecting reflectance data at lakes and rivers to build a larger library of high-resolution spectra under a variety of conditions. In addition, LandSat imagery is collected at 1-m resolution and is currently being evaluated by staff at NOAA for its utility at detection of blooms in smaller systems. It would be useful to compare the efficacy of these two approaches to decide on what will work best for the State.

A number of new methods are also emerging for analysis of cyanotoxins including using polymerase chain reaction (PCR) and other molecular technologies, pattern recognition software, and other technological advances. In order to make best use of these methods, they need to be evaluated for inclusion in the SWAMP laboratory methods guidance, and appropriate quality assurance measures need to be developed to guide their use. The current use of Quantitative PCR (qPCR) in Ohio as well as pilot studies in California will provide useful data for its possible future inclusion as a toxin screening method.

There are a number of mitigation and remediation methods described for cyanoHABs, and there may be a need to evaluate the efficacy of some of these methods and test new ones. For example, research may be necessary to evaluate reductions in nutrient loading associated with treatment methods, impacts on bloom severity and frequency, and most appropriate conditions for application of the method. This information will be included in the remediation guidance as it is updated periodically.

G. Outreach and Education

An ***Outreach and Education Program*** has not been funded at the time of this writing but should be developed and geared toward citizens, policymakers, health care professionals, veterinarians, and public agencies (such as city municipalities, county health, and environmental management agencies). There is a critical need to increase public awareness of HABs in order to increase recognition, public safety, and timely reporting of instances of HAB blooms or associated events. The Outreach and Education Program should adapt the training materials (from the Training component of the infrastructure), including guidance documents and HAB background materials, to educate the general public and policymakers. Much of the training material can be introduced in workshops and webinars, with webinar presentations posted on the centralized website, along with the training materials. Other states, in particular Florida, have excellent outreach and education programs that can serve as models.

Other educational tools that should be developed include ***factsheets*** on HABs and HAB specific social media sites. Several factsheets already exist through USEPA R9 (http://www2.epa.gov/region-9-documents/harmful-algal-blooms-questions-and-resources), the California Harmful Algal Bloom Monitoring and Alert Program (HABMAP; http://www.habmap.info/documents.html), the Southern California Coastal Water Research Project (http://www.sccwrp.org/Documents/FactSheets.aspx), University of California Santa Cruz (Kudela Lab; http://oceandatacenter.ucsc.edu/home/outreach/CyanoHAB.pdf), and the City of Watsonville's Pinto Lake program (http://cityofwatsonville.org/public-works-utilities/pinto-lake-park/help-save-pinto-lake/issues-facing-pinto-lake). Many states use ***social media*** applications to communicate with the public as to where blooms are occurring, and where waterbodies are posted or closed due to HABs. In addition, there are municipalities that use social media sites, such as Facebook and Twitter, to post information for the public, a practice that could be adopted for California. A successful outreach program should incorporate multiple forms of media to reach the largest number of people about HAB risks and events.

Another important aspect of outreach is developing the **protocols** needed to communicate about blooms and coordinate when blooms occur. Contact lists need to be developed that include lake managers, environmental health department staff, tribes, and water purveyors. This could be done at a statewide or regional level. Regional workshops could be held to introduce members of the network and to establish protocols for communication and coordination.

V. Potential Funding Mechanisms

While the resources have been provided to build most (but not all) of the infrastructure required to implement this Strategy by the SWAMP Freshwater HABs program (see Figure 1), long-term resources and funding mechanisms need to be identified to maintain the program and fund the remaining components.

Approaches in Other States: There are several approaches used by other states to maintain HAB monitoring programs that include a combination of the following mechanisms:

- Consistent funding through legislation
- Designated funds through state, county, or local agencies
- State mandated, fee-based funding
- Leveraged resources provided by existing monitoring programs or volunteer citizen scientist based programs
- Grants from federal (USEPA, NOAA, CDC, FDA) or state funding programs

The program in Washington combines state mandated, fee-based funding, consistent funding through legislature, and grants from federal programs in order to maintain HAB monitoring. Their marine and freshwater HABs programs were initially established with federal funding through a NOAA grant that laid the groundwork and built the infrastructure required for program initiation. The Washington State Legislature established the Freshwater Algae Control Program (currently called the Aquatic Algae Control Program). The funding for this program comes from $1 vessel registration fees which generates $540,000 per biennium. The Washington State Department of Ecology manages the program and budgets funds for laboratory costs of event response samples (algal taxonomic identification and toxin analysis) ($60,000 per year), the staff time to facilitate and coordinate the program, and annual grants to state and local government for freshwater algae projects (approximately $150,000 per year). This type of integrated interagency monitoring program also establishes cooperation between federal, state, and local health agencies.

Vermont's Lake Champlain monitoring program is leveraged off an existing nutrient TMDL monitoring program, but the initial funding for the HABs component (i.e. purchases of equipment and supplies, taxonomic and toxin analysis training etc.) was provided through a federal grant, and partnership with the academic community. The TMDL monitoring program collects HAB samples and the sample analysis is funded through designated State funds from the Department of Health.

Some states have freshwater HAB monitoring programs that are based on volunteer monitoring and therefore the funding required to maintain the program is minimal. New York's program is leveraged on an existing, volunteer-based, lake water quality monitoring program (Citizen Statewide Lake Assessment Program), which samples for water quality parameters on a routine basis. The Department of Environmental Conservation received funding from several federal grants to support sample analysis, with trained citizen scientist volunteers collecting samples. Since there is no formal funding or staff resources provided by the state agencies, the State's partnership with academic scientists has been instrumental to the program's success.

Still other states, such as Florida, perform the monitoring through the state agencies, Department of Environmental Protection, Department of Fish and Wildlife, Department of Health, and the water districts and health agencies. The funding for sample analysis comes from designated funds from state agencies. However, Florida's guidelines for posting waterbody advisories and public notification only require that there is a visible bloom; managers are not required to wait for sample results to determine if the bloom is toxic. This provides more flexibility with sampling and decreases the need for rapid event response. Florida also has the most extensive online tracking effort which allows public health professionals, environmental scientists, and managers to access the data and bloom reports in real-time.

Approaches for California: California will likely use a combination of approaches to fund this monitoring and assessment strategy for freshwater HABs. Funding mechanisms may include the following: 1) CWA Section 106 funds from SWAMP; 2) Water Bond grants; 3) other legislation; 4) redirection of existing funds; 5) Fees or taxes; 6) local and tribal agency contributions; 7) leveraged programs; and 8) volunteer activities.

Current SWAMP funding going to HABs monitoring comes from EPA ***Clean Water Act Section 106 funds***. Currently, three years of funding have been approved (2014 - 2017). While this funding may continue in some form in future years, there are several other SWAMP monitoring programs competing for this resource. As the cost of monitoring increases each year, the amount of monitoring this fund can support diminishes. In addition, the funds currently provided do not fully support the proposed elements of this strategy. Therefore, additional funds will need to be identified.

Grant funding would be particularly useful for building program infrastructure, developing approaches for risk assessment or for mitigation and remediation projects. California has periodically approved sizeable ***water bonds***, including the Water Quality, Supply, and Infrastructure Improvement Act of 2014 (Proposition 1). Proposition 1 does not call out specific funding for toxic algae blooms. However, there are a number of grant programs funded by Proposition 1 and managed by various agencies that could potentially be applicable. These grants typically can be expected to fund projects for a period of approximately 3 years. There are several programs that could be considered, for example, the Department of Fish and Wildlife's "Watershed Restoration" and "Delta Water Quality and Ecosystem Restoration" grant programs may be appropriate sources. One consideration is that most grant programs are focused on implementation rather than on monitoring. In the future, participating CCHAB

members who are not precluded from lobbying should work with legislators to ensure that specific language is included in water bonds related to funding of projects to manage and track toxic algae blooms.

Federal 319 grants are also available and could be another viable option, particularly for mitigation and remediation projects in water bodies with recurrent blooms. Many of the existing efforts to establish monitoring programs in individual waterbodies, or manage blooms, in California have been funded by grants. However, because grant funding is short-lived, it is not a stable basis for long term monitoring activities.

New ***legislation*** could be developed and implemented to fund or facilitate funding of monitoring, management, and education and outreach activities. In particular, Assembly Bill 300 (Monning) was submitted in 2015, requiring the SWRCB to establish and coordinate an "Algal Bloom Task Force" to assess and prioritize actions and research needed to prevent or mitigate blooms, and to make recommendations on funding, prevention, and long-term mitigation. This bill was in the Senate Appropriations Committee in October 2015 but has since been suspended. If it becomes law, it will help direct funds from other sources (such as Proposition 1) towards research, projects, and programs recommended by the Task Force. It does not provide funding directly. Other legislation could be developed to do so, for example through instituting ***fees or taxes*** on vessel registration (e.g. Washington), fertilizers, etc. Development of legislation is an activity which cannot be undertaken by state agency staff.

Without new legislation, stable state program funding could be obtained through **redirection of state funds** currently used for other purposes. Clearly, this has its disadvantages, in that programs are originated for specific and necessary reasons, typically have dedicated funding, and are addressing their own mandates. However, if cyanobacteria blooms continue to increase in size and frequency, the urgency associated with protecting public health and the environment may demand reprioritization of resources.

Local agencies and tribes are already contributing significantly to bloom management and monitoring. For example, in response to a nutrient TMDL, Lake County monitors Clear Lake and, in coordination with other stakeholders, has developed a monitoring and implementation plan. The Klamath Basin Monitoring Program is a coordinated, multi-organizational effort that includes basin-wide water quality monitoring, a data portal, and a plan for long-term stewardship, protection, and restoration of beneficial uses of the Klamath watershed. As blooms develop in waterbodies within the jurisdictions of local agencies, local response will be needed and local resources may be tapped. A major purpose of the infrastructural program elements developed through this strategy is to support these efforts, minimize costs, and maximize efficiencies in bloom response.

There are already a number of examples in California of **leveraging programs** for efficient monitoring. For example, the SWAMP Stream Pollution Trends (SPOT) program has included monitoring for cyanotoxins at integrator sites at the bottoms of major watersheds throughout the State of California. The SWAMP Perennial Streams Assessment (PSA) program has included

toxin producing taxa and toxin analysis in its probabilistic assessment of benthic algae. The Southern California Stormwater Monitoring Coalition has included similar sampling in its watershed assessment projects. The Southern California Coastal Water Research Project has leveraged monitoring by member agencies to learn more about the distribution of toxins and toxin producing taxa. Agencies and organizations participating in management and monitoring of cyanoHABs should continue to make use of their own monitoring program infrastructure, along with SWAMP products developed as part of the Freshwater HABs Program, to make cyanobacteria and toxin monitoring cost effective.

In California, the Marine Biotoxin Monitoring Program (facilitated by the CDPH, Division of Drinking Water and Environmental Management) is dependent on ***volunteers*** to monitor shellfish toxin levels and toxin producing marine algae. The volunteers conduct weekly monitoring, sample collection, and data upload, which allows the funding requirements for the program to be kept at a minimum. The program was initially established through FDA emergency response funds, and the coordinator position and sample analysis are the only components that require annual funds. This program provides an excellent example of how limited resources can be stretched by effective use of volunteer assistance. Volunteers are an important part of other existing programs, such as the Klamath Basin Monitoring Program.

VI. Partners Roles

Figure 1 illustrates the components of the assessment and support strategy framework that are mostly funded through SWAMP, partially being performed by other local, regional, state, or federal agencies, and those not currently being addressed. The purpose of this chapter is to identify the components of the framework that are not being addressed, but need to be, and to identify the agencies that have roles and responsibilities related to these tasks. It is not the intention of this document to assign tasks, but to match the tasks that should be performed with those normally performed by these agencies, in order to coordinate and implement a robust freshwater HABs program in California. Although coordination will provide funding efficiencies, additional funding (see previous chapter) must be accessed in order for agencies to take on these tasks. CCHAB would be the best forum for discussing and establishing these roles.

HABs and associated toxins relate directly to the missions of a wide range of agencies in California such as those dealing with human and wildlife adverse health effects, recreational impairments, and water supply; therefore, there is a broad base of agencies and user groups for which the Assessment and Support Strategy is relevant. Coordinating framework tasks with the mission and role of each agency will lead to efficient use of all resources directed at HAB monitoring and mitigation throughout California.

The agencies in California whose missions involve the protection of public and wildlife health and beneficial use impairments include (but are not limited to): SWRCB, Regional Water Boards, OEHHA, CDFW, CDPH, CDWR, USEPA, USGS, USFWS, Tribal Governments, cities and municipalities, and local and county health departments. Partnerships should be established

between these agencies and other water quality or HAB monitoring programs to monitor for HABs, either routinely or during events (such as the Eel River Recovery Project volunteer monitoring program for cyanotoxins, Klamath Basin Water Quality Monitoring Program).

Below is a list of tasks identified by the Strategy that need to be performed, or performed more fully, and the agencies that have related responsibilities. See Figure 1 and the section in the document that corresponds with the particular task for a full description.

Immediate Event Response for individual waterbodies is usually performed by agencies responsible for those waterbodies in consultation with local (county or city) environmental health departments. Responsible parties can include waterbody managers, parks departments, drinking water agencies, or environmental health departments. Infrastructure developed by SWAMP will assist these agencies in responding to cyanobacteria blooms, however, there needs to be a clear chain of command and responsibilities established for initiating and conducting monitoring, as well as alerting the public and other agencies. Currently, SWAMP has contracted with the SFEI to monitor waterbodies larger than 100 hectares (~250 acres) through satellite imagery, and to contact waterbody managers when a cyanobacteria bloom is detected. In addition, surveillance of especially high risk waterbodies should be conducted throughout the bloom season so that blooms can be detected in the early stages.

In the Klamath and Eel Rivers, groups have been formed that include waterbody managers, environmental health departments, the Regional Water Quality Control Board (Regional Water Board), tribes and volunteers to perform these tasks. Data collection to support listings and mitigation could be performed by these agencies/groups with assistance from the Regional Water Board in that area.

Long Term Event Response for individual waterbodies will also be supported by SWAMP infrastructure. Satellite monitoring and development of a data management system and website have been contracted to SFEI. The same entities involved in immediate event response should be involved in long term response. Since the responsibility of Regional Water Boards is to develop listings for impaired waterbodies and remediation strategies, including TMDLs, Regional Water Boards should be one of the lead agencies. Nutrient TMDLs being developed should be designed to protect against cyanobacteria blooms. Local Action Plans should be developed by local waterbody management agencies in collaboration with other involved agencies and tribes, and should have a process for public input and sharing data.

Field Assessment and Ambient Monitoring of individual waterbodies or watersheds should be performed, particularly during periods when blooms would be most likely to occur (e.g. warmer weather, longer light periods, and droughts). The same entities that respond to HAB events, both short and long term, on an individual waterbody could also be involved in ambient monitoring. However, other agencies that normally monitor these waterbodies could also conduct HAB monitoring (such as the CDWR or regional SWAMP programs).

Field Assessments and Ambient Monitoring at the State or Regional Scale are being conducted by a multitude of agencies (see Field Assessment and Monitoring section III.B.) by adding

cyanobacteria parameters to existing monitoring programs. Programs currently being developed, such as the Delta RMP, should include cyanobacteria and associated toxins in their list of analytes. Monitoring programs should consider the appropriate temporal and spatial scales necessary to effectively monitor cyanobacteria and cyanotoxins.

The use of satellite imagery is a way of monitoring cyanobacteria blooms that captures the temporal and spatial scale needed to assess blooms in waterbodies. This type of monitoring is being conducted by SWAMP, through SFEI, and may be conducted by CyAN, a national program, after 2020 (see section IV. A. - Satellite monitoring). However, additional resources are needed to extend and communicate this information. SWAMP satellite monitoring is only funded through 2017.

Assessing Risk at State and Regional Scale will partially be completed by an historical analysis of satellite and field data from 2002-2012 being conducted by SFEI and funded through SWAMP. Similar analyses should be conducted on a regular basis, such as every 10 years. A landscape risk analysis could be conducted by various agencies; however, this analysis could be very resource intensive and may not be predictive. Waterbodies that are indicated to be high risk through these analyses should be monitored on a regular basis.

Applied research and tool development is needed so the best tools and methods for detecting, quantifying, and remediating cyanobacteria blooms can be used. Three important applied research needs at this time are: 1) additional satellite imagery analysis for detection of blooms in waterbodies smaller than 250 acres; 2) improvements to methods for quantifying toxins in blooms, especially in turnaround time; and 3) improvements to methods for remediation and mitigation of blooms. Currently, federal agencies working on CyAN, as well as academic researchers, are improving remote sensing capabilities so that blooms can be detected more quickly and in smaller water bodies. Researchers at private companies and public universities are working on methods to decrease the time in which valid quantifiable results can be obtained from water and bloom samples, such as gene detection through qPCR. There is also work being conducted, especially in Australia, South Africa, Europe and China, to improve the mitigation and remediation methods for blooms. The Central Coast Regional Water Board has been awarded federal 319 grant funds for a mitigation/remediation project in Pinto Lake, Watsonville. In the future, there will probably be additional areas of research that will require funding and support.

Outreach is not being performed by any agency and is one of the highest priority tasks in this strategy. In 2015, there were dog deaths at Lake Chabot (San Leandro), on the Russian River, and the Sacramento River that appeared to be associated with cyanotoxins in these waterbodies. In each case, there was confusion regarding the course of action and how to alert the public. Guidance documents and training, being developed by SWAMP and CCHAB, will help to develop clear, standardized procedures to follow when a bloom is identified or a death or illness that seems to be related to a bloom occurs. However, the public, responsible agencies, veterinarians, and health care professionals need to be informed about cyanotoxins to perform their role effectively.

The Department of Public Health's Environmental Health Investigation Branch (EHIB) has previously conducted education and outreach on fish mercury contamination in the Delta through the Fish Mercury Project (FMP). Under FMP, EHIB conducted needs assessments with community-based organizations representing diverse fishing populations, as well as with other stakeholders. The needs assessments included a variety of tools such as focus groups, meetings, interviews, etc. They also formed a stakeholder advisory group to provide input on project activities, funded community-based education projects through a grants program, conducted training, provided technical assistance, held two public forums, and developed multilingual materials. In addition, they evaluated the comprehension of advisory messages through interviews with fish consumers. Currently, EHIB is developing signage for cyanobacteria blooms that will be used by CCHAB in their guidance. EHIB has the experience and, with funding, a cyanotoxin education and outreach project could be enacted. Frequently Asked Questions (FAQs) have already been developed by USEPA Region 9 and others that could be used for this project.

Another part of outreach is developing the protocols needed to coordinate and communicate about blooms. Several groups including SFEI, California Association of Lake Managers (CALMs), and the North Coast Regional Water Quality Control Board are currently developing a list of contacts for bloom notification based on satellite imagery, to coordinate and communicate about blooms. The list should include lake managers, environmental health departments, water purveyors, and tribes. Regional Water Boards could facilitate this process by holding regional workshops to introduce members of the network and to develop protocols for communication and coordination. At the time of this writing, the North Coast Regional Water Quality Control Board is embarking on this process. Their process could be used as a model by other Regional Water Boards for developing regional networks.

VII. Strategy Review

With our understanding of CyanoHABs and the associated health risk to the public, pets, wildlife, and livestock continuing to evolve, the monitoring and assessment framework should be re-evaluated every 5 years by the CCHAB network to determine if the existing goals are being met, and to determine if there are any additional objectives that need to be included. Areas of program success should be highlighted in the amended document and any program weaknesses should be discussed and addressed when the Strategy is reviewed and revised.

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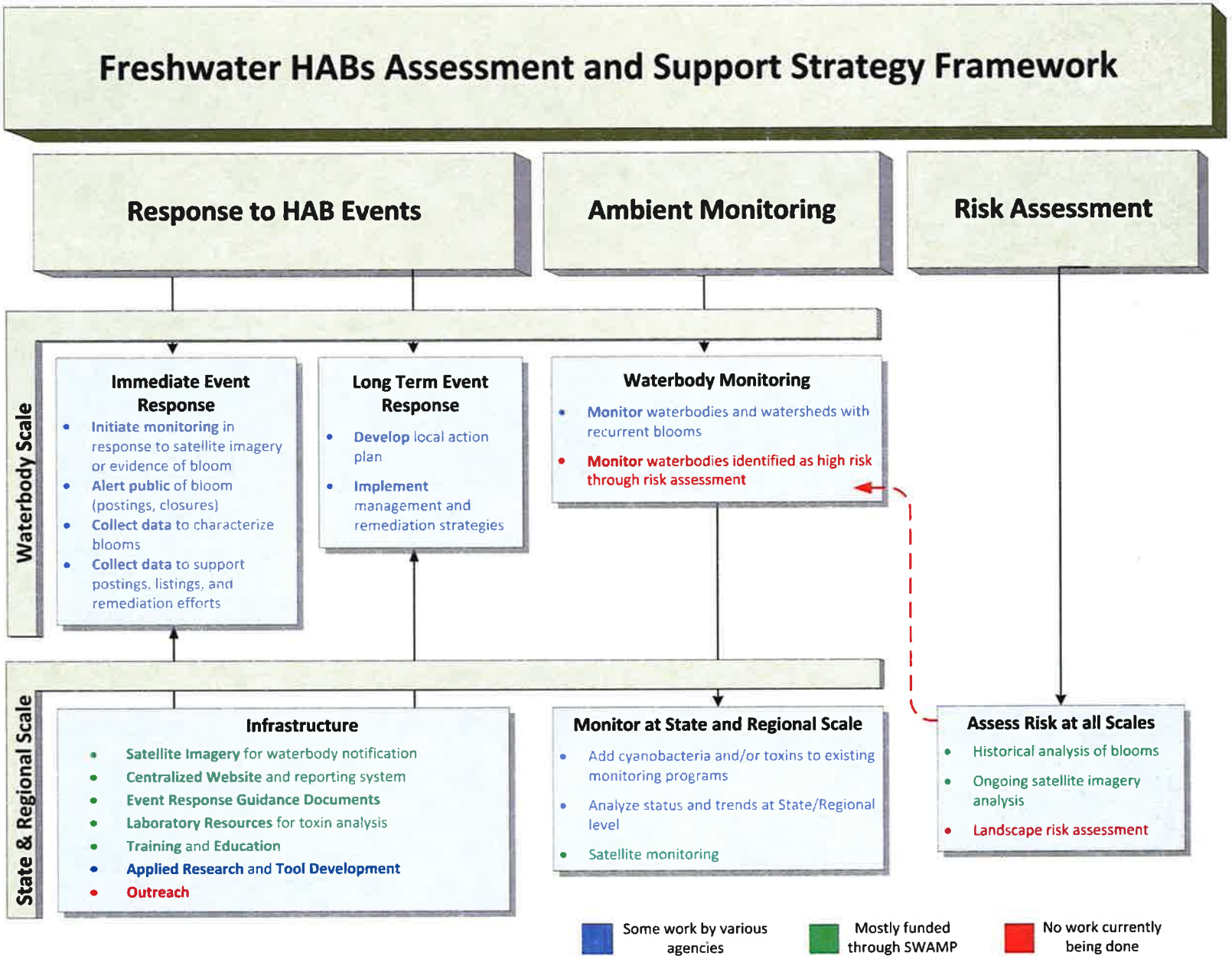


Figure 1. Freshwater HAB Assessment and Support Framework

Figure 2. Infrastructure needed to support the Assessment and Support Framework

Appendix A. Background on Harmful Algal Blooms

At the base of the food chain in fresh, brackish, and marine systems are photosynthetic cyanobacteria and algae. Both single-celled, microscopic and larger, multicellular forms exist. These cyanobacteria and algae provide organic matter and energy to higher trophic levels. Under certain environmental conditions, a rapid increase or accumulation of microscopic algae can occur, and "harmful algal blooms" (HABs) may result, that can have negative impacts on the environment, people, pets, wildlife, or livestock, as well as the economy. The harmful mechanisms can be related to chemical effects (the production of toxins), biochemical effects from biomass accumulation (anoxia, hypoxia, habitat alteration), or physical features (spines that cause gill irritation). The main focus of this strategy is on freshwater HABs that produce toxins, although these toxin-producers may also be found, or cause issues, in brackish and marine environments (see Appendix B for regional concerns in California).

The most researched group of freshwater HABs is the cyanobacteria, or blue-green algae. These are problematic because they can impede recreational and beneficial uses of waterbodies by reducing aesthetics, lowering dissolved oxygen concentration, causing taste and odor problems in drinking water, and producing potent cyanotoxins, associated with illness and mortality in people, pets, livestock, and wildlife. Cyanobacteria blooms and their associated toxins have increased globally in geographic distribution, frequency, duration, and severity (Chen et al., 1993; Dawson; 1998; Amorim and Vasconcelos, 1999; Domingos et al., 1999; Lehman et al., 2005; Guo, 2007; Paerl and Huisman, 2008; Hudnell, 2010; Paerl and Paul, 2012). Non-cyanobacteria HAB events have also increased, the most common of which is the golden haptophyte alga, *Prymnesium parvum*, which has caused fish kills in the east, mid-west and southern states, and recently in Southern California, resulting in the impairment of beneficial uses of recreational lakes.

There are a large number of environmental factors that have been linked to bloom increases and toxin production (reviews by O'Neil et al., 2012; Paerl and Otten, 2013). These include climate change, nutrient over-enrichment, temperature, salinity, water residence time, vertical stratification, organic matter enrichment, and high pH (Paerl, 1988; Shapiro and Wright, 1990; Paerl, 1996; Paerl and Fulton, 2006; Carmichael, 2008; Paerl and Huisman, 2009; Paerl et al., 2011; O'Neil et al, 2012; Paerl and Paul, 2012; Paerl and Otten, 2013). The specific nutrients controlling cyanobacteria blooms have been debated in recent years. Historically, phosphorus has been the primary nutrient attributed to controlling cyanobacteria blooms in freshwater systems. However, recent studies have shown that nitrogen also controls cyanobacteria blooms, so that both nitrogen and phosphorous, and their ratio, need to be considered in water quality management strategies (Conley et al., 2009; Scott and McCarthy, 2010; Xu et al., 2010; Paerl et al., 2011; Wilhelm et al., 2011; Paerl and Otten, 2013).

Appendix B. California Regional CyanoHABs

There are several cyanobacteria "hot spots" in California where blooms are recurrent and, as a result, monitoring programs have been established. These areas include the Klamath Basin, Pinto Lake and Monterey Bay, San Francisco Bay area and Delta, Clear Lake and Southern California (Figure 3).

Klamath Basin

The Klamath Basin Water Quality Monitoring Program is the most well-established routine monitoring program in the State. Funded through the Klamath Hydroelectric Settlement Agreement, this monitoring is part of a larger effort organized by members of the Klamath Basin Monitoring Program, which includes a basin-wide water quality monitoring and coordination program, data portal, and a plan for long-term stewardship, protection, and restoration of all beneficial uses within the watershed. While a variety of toxin producing species have been documented in the watershed, (such as *Aphanizomenon flos-aquae, Anabaena circinalis, Gloeotrichia echinulate,* and *Oscillatoria* sp.) samples annually have had high cell densities of *Microcystis aeruginosa* and high concentrations of its toxin, microcystin, since 2004 (Kann, 2004; Jacoby and Kann, 2007; Fetcho, 2007; Moisander et al., 2009). *Microcystis aeruginosa* cells and microcystin have been documented in mussels (bivalves) and fish tissue collected from the river (Kann, 2008).

The Klamath River has been listed as impaired under the Clean Water Act section 303(d) due to excessive concentrations of microcystins. The highest concentrations of both *M. aeruginosa* cells and microcystin occur in Copco and Iron Gate Reservoirs, but have been detected as far downstream (200 river miles) as the Klamath River Estuary (Otten et al, 2015; Yurok Tribe Environmental Program 2007). To understand the sources and environmental stressors that drive microcystin, and other 303(d) listed impairments, many organizations coordinate monitoring at a number of reservoir and river sites throughout the basin for water quality parameters (turbidity, temperature, dissolved oxygen, nutrients, etc.) as well as for microcystins and algal species enumeration. (http://sfei.maps.arcgis.com/apps/MapSeries/index.html?appid=9b10920b676b4dfebce14f8c4ea70c4d&entry=1)

Pinto Lake and Monterey Bay

Monterey Bay is an area that has also been well studied in recent years. The mortality of over 30 endangered California Sea Otters (*Enhydra lutris*) in Monterey Bay was determined to be due to microcystin intoxication, with ingestion of contaminated marine bivalves suggested as a primary mechanism (Miller et al., 2010). Pinto Lake, a eutrophic lake that experiences frequent cyanobacteria blooms and drains to Monterey Bay via the Pajaro River, was identified as the primary source of the toxin (Miller et al., 2010; Kudela, 2011). Microcystin-laden water from the Pajaro River, and other tributaries to the Bay, flow to the coast where the toxin is biomagnified by bivalves, and ultimately consumed by otters (Miller et al., 2010). In tank studies, microcystins have been shown to bioaccumulate in commercially and recreationally-harvested

invertebrates such as Pacific oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*) (Miller et al., 2010).

Microcystins were shown to be present and persistent in most of the coastal watersheds that flow to the Monterey Bay National Marine Sanctuary from a 3 year time-series survey (Gibble and Kudela, 2014). The survey showed seasonal toxin patterns with highest concentrations in the autumn and spring and concluded that microcystins are a persistent issue in this area. Nutrient loading was determined to be a significant predictor of microcystin concentrations in the watersheds (Gibble and Kudela, 2014). These studies have shown cyanotoxins to have far reaching effects downstream of their origin, and have promoted cyanotoxins from predominantly a freshwater issue to a land-sea interface problem.

San Francisco Bay Area and Delta

Microcystin contamination of the San Francisco Bay and Delta ecosystem has shown similar seasonal characteristics as Monterey Bay (Lehman et al., 2005; Lehman et al., 2008; Moisander et al., 2009), and there is evidence for increasing blooms with climate change (Lehman et al. 2013). Blooms of *Microcystis aeruginosa* have been documented since 1999 in the Delta, and blooms of *Aphanizomenon* sp. and presence of *Anabaena* sp. have also been documented routinely (Lehman and Waller, 2003; Lehman et al., 2010; Mioni et al., 2012). Cyanobacteria blooms have been identified as an impairment in the Delta, and the Central Valley Regional Water Quality Control Board is currently developing a science plan for the Delta on nutrient management policies that consider cyanobacteria bloom management.

Several man-made lakes in the East Bay Regional Parks (eastern San Francisco Bay area) are severely impacted by cyanobacteria blooms, including Lake Temescal, Lake Chabot, and a few others. Bloom severity in recent years has been such that lakes have been closed at times for swimming and contact sports. At least three dog deaths have been linked to these blooms. Monitoring by the East Bay Regional Park District is conducted in response to visual identification of scums and other evidence of blooms.

Clear Lake

Clear Lake has recurring cyanobacteria blooms that have impaired lake beneficial uses, including recreational activities, wildlife habitat and most importantly, drinking water. It has been listed on the 303d list of impaired waterbodies since 1986; however, efforts to reduce phosphorus loads and sediment cycling have failed to decrease cyanoHABs in recent years (Mioni et al., 2012).

Southern California

In Southern California, a number of screening assessments have documented cyanotoxins in multiple waterbody types, including depressional wetlands, lakes, reservoirs, coastal lagoons, and estuaries (Fetscher et al., 2015). A probabilistic survey conducted in the spring in depressional wetlands indicated that microcystins were detected at 25% of tested sites from 2011-2013. Another San Diego based study focused on lakes, estuaries, lagoons, and reservoirs

in 2013; passive samplers (solid phase adsorption) detected microcystins at every site, and traditional discrete ("one-time grab") samples were found to underestimate the prevalence of toxin and miss toxic events. Similar results were found in studies of Pinto Lake at weekly timescales (Kudela, 2011).

In 2014, there were so many cyanoHAB blooms in coastal habitats reported to the San Diego Regional Water Board that an *ad hoc* field survey was conducted of estuaries, lagoons, lakes, and reservoirs. Microcystins were detected at several lakes at varying concentrations and microscopic examination of water samples indicated multiple, potentially toxic species at the sites sampled. Four lakes in Riverside, CA were sampled in the spring of 2014 and multiple toxins were detected simultaneously, including cylindrospermopsin, anatoxin-a and microcystins, with several samples containing cyanotoxin concentrations above recreational action level thresholds (see Appendix C for OEHHA action level thresholds). Additionally, four lakes in the Los Angeles and Orange County areas have experienced costly fish kills, attributed to blooms of the toxin producing golden algae, *Prynmesium parvum*.

Figure 3. Areas in California with recurrent blooms and ongoing monitoring activities

Appendix C. Toxin Thresholds

In June, 2015, the USEPA released health advisory guidance for algal toxins in drinking water in order to protect human health. The recommended 10 day health advisory values are 0.3 µg/L for microcystin and 0.7 µg/L for cylindrospermopsin for children younger than school age (values are 1.6 µg/L for microcystin and 3.0 µg/L for cylindrospermopsin for all other ages).

OEHHA has recommended health-based toxin exposure thresholds (also known as "action levels") to protect humans, pets, and livestock during recreational exposure for three cyanotoxins (microcystins, cylindrospermopsin, and anatoxin-a). These health-based exposure thresholds are summarized in Table 1, and published in the "Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins" (OEHHA, 2012; http://www.oehha.ca.gov/risk/pdf/cyanotoxins053112.pdf). Action levels have also been developed for fish and shellfish consumption. These exposure thresholds are levels at which no health effects are anticipated, and indicate additional action (i.e. monitoring) may be advised.

The CCHAB Voluntary Guidance document, "Cyanobacteria in California Recreational Water Bodies: Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification" is currently being revised to update the toxin exposure thresholds for posting advisories and warnings.

Table 1. OEHHA Action Thresholds for cyanotoxins in California (from OEHHA, 2012)

	Microcystins (LA, LR, RR, and YR)	Anatoxin-a	Cylindrospermopsin	Media (units)
Human recreational uses[1]	0.8	90	4	Water (µg/L)
Human fish consumption	10	5000	70	Fish (ng/g) ww[2]
Subchronic water intake (dog)[3]	2	100	10	Water (µg/L)
Subchronic crust and mat intake (dog)	0.01	0.3	0.04	Crusts and Mats (mg/kg) dw[4]
Acute water intake (dog)[5]	100	100	200	Water (µg/L)
Acute crust and mat intake (dog)	0.5	0.3	0.5	Crusts and Mats (mg/kg) dw[4]
Subchronic water intake (cattle)[6]	0.9	40	5	Water (µg/L)
Subchronic crust and mat intake (cattle)[6]	0.1	3	0.4	Crusts and Mats (mg/kg) dw[4]
Acute water intake (cattle)[6]	50	40	60	Water (µg/L)

[1]The most highly exposed of all the recreational users were 7- to 10-year-old swimmers. Boaters and water-skiers are less exposed and therefore protected by these action levels. This level should not be used to judge acceptability of drinking water concentrations.

[2]Wet weight (ww) or fresh weight

[3]Subchronic refers to exposure over multiple days

[4]Based on sample dry weight

[5]Acute refers to exposures in a single day

[6]Based on small breed dairy cows because their potential exposure to cyanotoxins is greatest

Appendix D. Abbreviations and Acronyms

CCHAB	California Cyanobacteria Harmful Algal Bloom Network
CDC	Centers for Disease Control
CDFW	California Department of Fish and Wildlife
CDPH	California Department of Public Health
CDWR	California Department of Water Resources
CEDEN	California Environmental Data Exchange Network
CyAN	Cyanobacteria Assessment Network, National
CyanoHAB	Cyanobacteria Harmful Algal Bloom
EHIB	Environmental Health Investigation Branch
ELISA	Enzyme-Linked ImmunoSorbent Assay
ESA	European Space Agency
FHAB	Freshwater Harmful Algal Blooms
FMP	Fish Mercury Project
GIS	Geographic Information Systems
HAB	Harmful Algal Bloom
HABMAP	Harmful Algal Bloom
LC-MS	Liquid Chromatography-Mass Spectrometry
MERIS	Medium Resolution Imaging Spectrometer
Monitoring Council	California Water Quality Monitoring Council
MQO	Measurement Quality Objectives
NASA	National Aeronautics and Space Administration
NOAA	National Oceanic and Atmospheric Administration
OEHHA	Office of Environmental Health and Hazard Assessment
PSA	Perennial Streams Assessment
RMC	Regional Monitoring Coalition (San Francisco Bay Area)
SFEI	San Francisco Estuary Institute
SMC	Stormwater Monitoring Coalition
SOP	Standard Operating Procedures
SPoT	Stream Pollution Trends
SWAMP	Surface Water Ambient Monitoring Program
SWAMP IQ	SWAMP Information Management and Quality Assurance Center
SWRCB	State Water Resources Control Board
TMDL	Total Daily Maximum Loads
USEPA	U.S. Environmental Protection Agency
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
Water Boards	Regional Water Quality Control Boards

Attachment 2

2019 Oregon Harmful Algae Bloom Surveillance (HABS) Program Recreational Use Public Health Advisory Guidelines Cyanobacterial Blooms in Freshwater Bodies

Oregon Harmful Algae Bloom Surveillance (HABS) Program

Recreational Use Public Health Advisory Guidelines
Cyanobacterial Blooms in Freshwater Bodies

Oregon Health Authority

Public Health Division
Center for Health Protection
Environmental Public Health Section

Updated May 2019

Recreational Use Public Health Advisory Guidelines for Cyanobacterial Blooms in Freshwater Bodies

Oregon Health Authority Public Health Division
Center for Health Protection

Table of Contents

Introduction

Cyanobacteria, also known as blue-green algae, are commonly found in many fresh and saltwater environments around the world. Some cyanobacteria species are referred to as toxigenic because they have the potential to produce toxins that can harm people, pets and wildlife.

Some Oregon water bodies are monitored for cyanobacterial harmful blooms (CyanoHABs). The number of waterbodies monitored is affected by available local, state, and federal resources and the costs associated with sampling and analysis. Historically the decision-making process for issuing and lifting health advisories varied according to the managing jurisdiction of a specific water body. In 2009, the Oregon Health Authority, Public Health Division (OHA) assumed responsibility for the decision-making process and for issuing and lifting public health advisories when CyanoHABs are detected.

The OHA is working to gain a better understanding about the occurrence of CyanoHABs in Oregon and their impact on human health. Funding for Oregon's Harmful Algae Bloom Surveillance program was through a five-year federal grant from the U.S. Centers for Disease Control and Prevention (CDC). That grant ceased in September of 2013. Currently program staff implement the highest priority activities such as the issuing and lifting of advisories with no dedicated funding.

OHA program objectives:

- Provide a single, statewide point of contact to all agencies and groups performing sampling and analysis
- Track freshwater CyanoHABs with data provided by partner agencies
- Track cases of human and animal illnesses related to CyanoHABs
- Enter environmental and health data for OHA tracking
- Build capacity of our partners to monitor water bodies in a scientifically sound manner with the goal of protecting public health
- Provide technical assistance to partner agencies to assess health risks associated with cyanotoxins
- Educate and inform the public regarding health risks due to CyanoHABs

Background

The recreational use public health advisory guidelines in this document were developed and are modified based on the most current national data and references, and on monitoring data received from our waterbody partners and stakeholders.

These guidelines are used to educate the public and our partners about how and when OHA issues and lifts recreational use public health advisories. Public health advisories help to inform the public of the health risks associated with exposure to potentially toxic cyanobacteria in Oregon's recreational fresh waters.

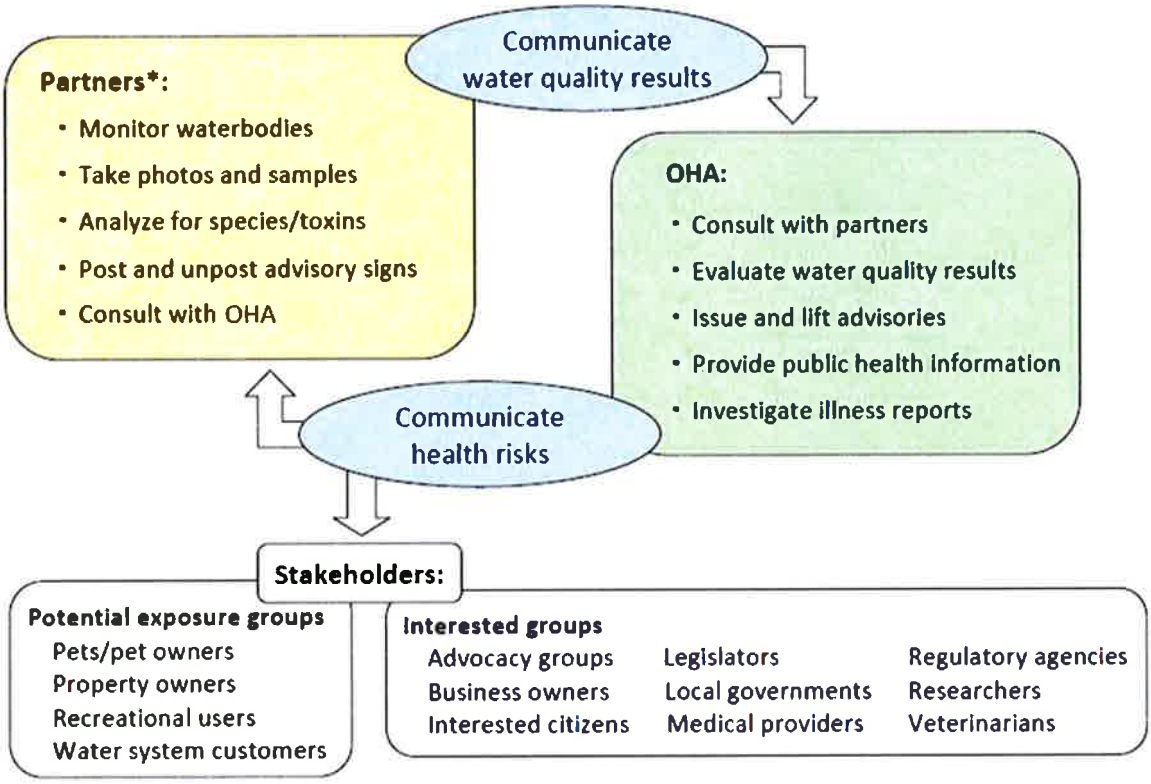
OHA authority for public health and safety fall under Title 36, Oregon Revised Statute (ORS), Chapter 431.035 to 431.530.

CyanoHAB Coordination Process

Specific actions are involved in monitoring, responding to and communicating information about CyanoHAB blooms.

Coordination among the OHA and its partners and stakeholders is paramount to complete the advisory process from identification, sampling and analysis of a bloom to notifying the public of a recreational use public health advisory. Figure 1 depicts the flow of activities among all entities involved in CyanoHAB incidents.

Figure 1. Activities involved in monitoring and responding to CyanoHABs



*Oregon Department of Environmental Quality, U.S. Forest Service, U.S. Army Corps of Engineers and other waterbody managers.

The main role of the OHA is to issue and lift health advisories based on water quality data provided by partners and to provide risk communication.

Partners in this effort include the Oregon Department of Environmental Quality, U.S. Forest Service, U.S. Army Corps of Engineers and other waterbody managers.

Stakeholders in the process are classified in two sub-groups:

- Exposure: Those with a greater risk of illness from cyanotoxins through recreational activities. The main routes of exposure are through ingestion and inhalation of affected water. Although cyanotoxins are not absorbed through the skin, people with sensitivities can develop a rash when coming into contact with a

CyanoHAB. More information regarding potential routes of exposure is provided in Appendix C.

- Interest: Those with varying levels of need, involvement or interest in program operations or policies, those affected by the program, or are intended users of program outcomes and findings.

Table 1. Roles and responsibilities for monitoring and responding to a CyanoHAB

Activity	*Lead role*	*Assist*
Monitor	Partners monitor water bodies through on-site observations for evidence of CyanoHABs	OHA provides guidance on how to monitor for public health purposes and in identifying cyanobacteria
Collect water samples	Partners use scientifically acceptable methods to obtain water samples	OHA provides guidance on sampling techniques
Analyze samples	Partners contract with laboratories that are qualified to perform the required analyses	OHA provides a list of laboratories with appropriate analytic capabilities
Issue or lift advisories	OHA evaluates data and compares test results to established criteria to determine if an advisory should be issued or lifted	Partners respond to questions about waterbody status
Communicate advisory information	OHA informs the public through advisory news releases, GovDelivery messages, broadcast and print media, a toll-free hotline, the HABs website and educational materials	Partners and local health departments inform constituents of health advisory status through news releases and signage
Ongoing communication between the OHA and partners occurs throughout the bloom season regarding advisory decisions, bloom information, water quality data and illness reports.		

Protocol for Issuing a Recreational Use Public Health Advisory

OHA is responsible for the decision-making and communication process of issuing and lifting recreational use public health advisories.

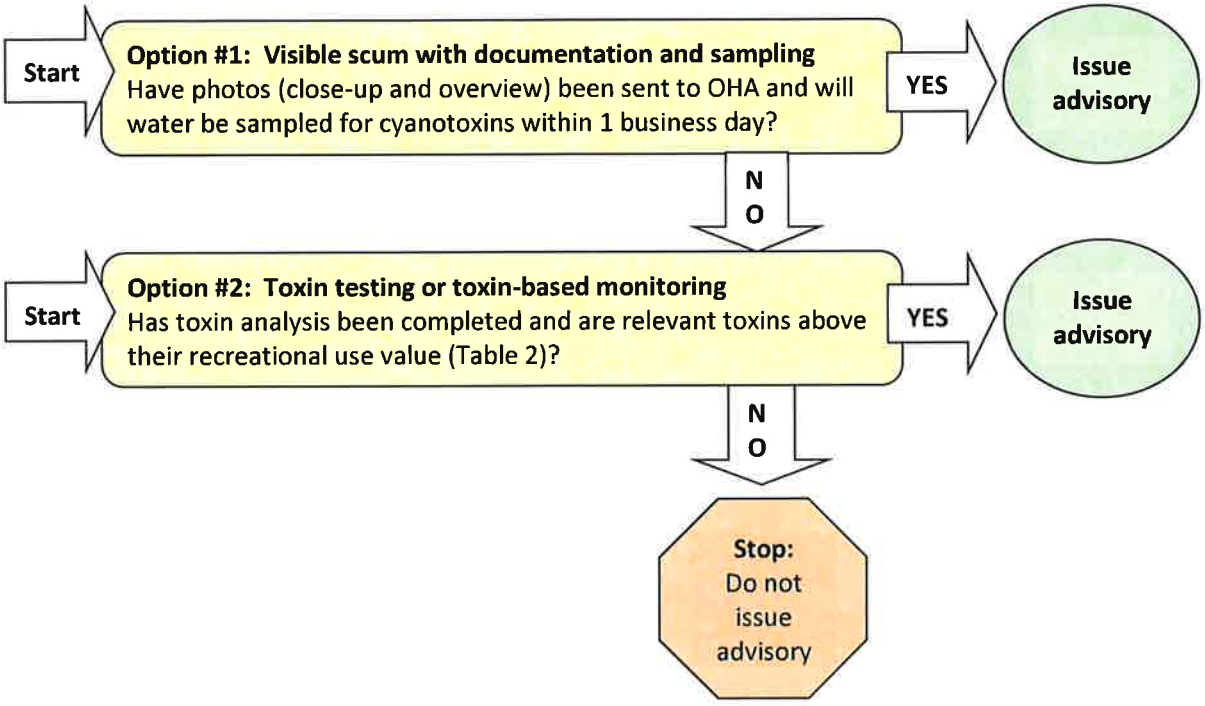
OHA criteria for issuing a public health advisory depend on the method selected by the water body manager. Options are:

- Visible scum with supporting photographs and toxin analysis within 1 business day
- Analysis showing cyanotoxin levels above OHA recreational use values (RUVs)

Scum is defined as a visible mass of cyanobacteria identified in the water body. Accumulations of greatest concern are those occurring at or near recreational access points.

The difference between Options 1 and 2 is the time between when the CyanoHAB is identified and when an advisory is issued. If Option 1 is used an advisory is issued as soon as visible scum is identified. If toxin analysis determines levels below OHA's RUVs, the advisory is lifted immediately. If levels are above, the advisory stays in place until additional toxin analysis shows levels below the RUVs. If Option 2 is used, an advisory would only be issued if cyanotoxin levels are above OHA's RUVs once data is submitted. Option 1 is used when waterbody managers are interested in a more health protective approach.

Figure 2. OHA process for issuing public health advisories for a CyanoHAB



OHA RUVs for cyanotoxins are based on information from the Environmental Protection Agency (EPA) and toxicological review of peer-reviewed scientific literature. More information about how OHA derived RUVs is provided in Appendix C. More information regarding the rationale used to help determine when advisories should be issued or lifted is provided in Appendix A.

Additional Guidance on the Toxin Based Monitoring Program: Option 2

Toxin testing provides the most accurate information in terms of protecting public health and results in health advisory decisions that are based on actual human health risk.

Because cyanobacteria do not always produce toxins, even when blooms are large, it is anticipated that Option 2 will result in fewer and potentially more targeted public health advisories for a given water body.

OHA's cyanotoxin RUVs listed in Table 2, are the basis for determining whether an advisory is issued. The OHA Sampling Guidelines document contains detailed information on how to conduct a toxin-based monitoring program.

Table 2. Health advisory RUVs for cyanotoxins in Oregon recreational waters (µg/L)

*RUVs**	*Microcystin*	*Anatoxin-a*	*Saxitoxin*	*Cylindrospermopsin*
	8	15	8	15

*See Appendix B for the detailed rationale behind these RUVs.

OHA has also developed dog-specific RUVs. They are for informational purposes only to educate pet owners about the susceptibility of dogs to cyanotoxins and are not used as a basis for issuing public health advisories. These RUVs can be found in Appendix C.

Note: While waiting for laboratory analysis to determine if a recreational use public health advisory should be issued, local water body management may post educational and/or caution signs as a precautionary measure, to alert the public of potential health risks associated with recreating in a water body during a CyanoHAB.

OHA has educational posters on the HAB webpage to use all year round, especially on waterbodies where blooms have been identified in the past. You can find an informational poster about blooms in Oregon here:
https://www.oregon.gov/oha/PH/HEALTHYENVIRONMENTS/RECREATION/HARMFULALGAEBLOOMS/Documents/HABSinOregon_FINAL_Web.pdf
There is also a poster created specifically for dogs in English:
https://www.oregon.gov/oha/PH/HEALTHYENVIRONMENTS/RECREATION/HARMFULALGAEBLOOMS/Documents/HAB-dog-safety.pdf
and in Spanish:
https://www.oregon.gov/oha/PH/HEALTHYENVIRONMENTS/RECREATION/HARMFULALGAEBLOOMS/Documents/HAB-dog-safety-sp.pdf

Aphanizomenon flos-aquae

Aphanizomenon flos-aquae (AFA) is a species of cyanobacteria commonly found in Oregon's fresh waters. Since 2012, studies have shown that AFA can produce cyanotoxins in other parts of the world, and current toxin testing of AFA here in Oregon has determined that toxins can be produced in Oregon waters where AFA is present. Given the uncertainty relative to the amount of toxin produced by AFA, OHA no longer supports the exclusion of AFA from the list of potentially toxigenic species used to determine which toxin tests to conduct. As before, other species of the genus *Aphanizomenon*, such as *A. gracile* have been demonstrated to produce cyanotoxins. Table B-1 in appendix B has a list of cyanobacteria found in Oregon and the toxins OHA recommends be analyzed for.

Advisory protocol for very large, geographically unique waterbodies

For these waterbodies OHA will, to the extent possible based on available data, tailor recreational advisories geographically on very large and unique lakes (e.g., Lake Billy Chinook, Upper Klamath Lake, Detroit Lake, Tenmile Lake, etc.) that lend themselves to partial vs. whole lake advisories. These tailored advisories can simultaneously provide protection of public health where risk is high, while allowing recreational activities to continue in unaffected areas where exposure is low. Tailored advisories will be evaluated by OHA on a case-by-case basis working with waterbody managers and using satellite imagery tools to inform the advisories issued.

Protocol for Lifting a Public Health Advisory

Table 3 on page 8 summarizes the lifting criteria for advisories issued based on the type of monitoring that led to the advisory.

Table 3. Criteria for lifting advisories

Monitoring option used to generate advisory	*Lifting criteria*
Option 1: Visible Scum	Cyanotoxin results from initial sample below RUVs
Option 2: Toxin based monitoring	Cyanotoxin results below RUVs AND either there is a commitment to continue bi-weekly sampling until bloom gone OR bloom is visibly gone
Toxin Based monitoring on waterbodies used for drinking water (and other scenarios where sampling is more frequent than bi-weekly)	When lab analysis from a second sample shows cyanotoxin results below RUVs. *Certain instances may require OHA to determine the number of consecutive samples necessary to lift (done on a case-by-case basis)*

Cyanobacteria can release their toxins during bloom formation and as the bloom is declining. Cyanotoxins, like microcystin and cylindrospermopsin can take some time to degrade even after a bloom has dispersed. It is possible therefore, for visual observations to indicate that a bloom has disappeared and still have toxins present. To reduce the risk of exposure to the public from lingering toxins, in all cases, toxin analysis must be completed to lift an advisory.

If an advisory is issued based on Option 1 (visible scum) and initial sample results verify that toxins are below RUVs, OHA will immediately lift the advisory. In this case OHA advises continued visual assessment of the bloom and resampling if a change in bloom condition or size is observed.

If an advisory is issued based on Option 2 (toxin results above RUVs) and testing is bi-weekly or less frequent, OHA will lift the advisory as soon as regular toxin testing indicates that total (intracellular and extracellular) toxin levels are below RUVs as long as there is a commitment to continue bi-weekly monitoring. In this case, even though the advisory has been lifted, OHA advises continued toxin-based monitoring every other week until the bloom is gone to ensure toxin levels remain below RUVs. If continued sampling shows an increase in toxins above RUVs, a second advisory would be issued. If sampling shows toxin levels are below RUVs and the bloom has visually dispersed, OHA will lift the advisory immediately.

We recommend contacting your lab for the most current cost of analyses and for preservation and shipping instructions for your sample. Be sure to choose a laboratory that can analyze for cyanotoxins produced by the cyanobacteria present (see Appendix B, Table B-1).

Lifting Protocol for Frequently Sampled Waterbodies

Permanent drinking water rules for cyanotoxin sampling and analysis will provide OHA with raw water analyses on a biweekly or more frequent basis throughout the season for susceptible water bodies used as drinking water sources. Concurrently, data from other waterbodies such as Upper Klamath Lake are submitted on a more frequent than normal basis as part of a monitoring partnership among tribes and local, state, and federal agencies.

Frequent sampling and analysis have confirmed the high variability of toxin levels during the life of a bloom. This variability can lead to the increased issuing and lifting of recreational advisories we call bouncing advisories. Bouncing advisories are resource intensive and can cause advisory fatigue. For these reasons, OHA has changed the recreational use advisory protocol for lifting advisories on frequently sampled waterbodies which will reduce the recurrence of advisories throughout the season.

When frequent sampling and analysis occur, OHA will determine on a case-by-case basis the number of consecutive samples necessary to lift a recreational use advisory. In most cases, an advisory will be lifted when lab analysis from a second sample shows that the cyanotoxins present continue to be below OHA RUVs.

Laboratories

Commercial laboratories use a variety of comparable methods currently available to analyze for cyanotoxins. When requesting toxin testing, ensure the lab uses a method detection level less than the RUVs in Table 2. Note: OHA will not accept field-ready test kits (dipsticks, etc.) for cyanotoxins as a basis for lifting an advisory. However, these kits may be useful for monitoring the progress of a bloom throughout the season.

Analysis can be costly depending on the method and equipment used. Lab staff can provide you with the most current cost of toxin analyses prior to submitting a sample. In general, the ELISA method is least expensive for determining levels of cyanotoxin in the bloom. ELISA methods are not currently available for anatoxin-a. However, Abraxis has introduced a micro-titer plate format (96T) receptor-binding assay (RBA) kit for anatoxin-a. The kit provides two protocols. The EZ protocol requires no sample preparation and has a range of 5 - 500 ppb. If a lower limit of detection is required, the enhanced sensitivity (ES) SPE sample concentration may be performed. This kit provides a real-time, economical, accurate and sensitive alternative for research and monitoring programs.

Note: All cyanobacteria produce lipopolysaccharides that can cause skin irritation, so there is no need to test for them.

Public Notification Methods

OHA uses several concurrent notification methods in the issuing and lifting of public health advisories. The specific methods are as follows:

Email: An email alert is sent to the following:

- Health department administrators and officials
- Tribal leaders and tribal health directors

News Releases: OHA issues statewide news releases which may be picked up and reported by broadcast and print media outlets across Oregon. These releases contain information about the nature and location of the advisory, possible health effects, recommended protective actions and where people can obtain more information.

GovDelivery listserv messages: A GovDelivery message is sent to notify members about a health advisory issue or lift immediately after the advisory news release is issued. List serv recipients can also choose to receive a text message as part of this notification process. Currently this listserv has nearly 6,000 members. OHA recommends subscribing to GovDelivery to receive real-time information about HAB advisories issued and lifted across the state. Subscribe to email alerts.

Program Website: The program maintains a website where advisory information is immediately posted, providing access to up-to-date information on the issuing and lifting of HAB advisories in Oregon. The public and others can also access resources for water samplers, prevention tips, frequently asked questions, and general information about CyanoHABs. The website is available at ***www.healthoregon.org/hab***.

Hotline: A statewide toll-free telephone service (877-290-6767) provides updated advisory information to the public, which is particularly helpful for individuals who are traveling, or those without Internet access.

Program Contact Information

Email: habhealth@state.or.us
Phone: (971) 673-0440, Toll Free: (877) 290-6767 and press 4
Website: ***www.healthoregon.org/hab***

Appendix A: Rationale used to determine when advisories should be issued and lifted for CyanoHABs

The use of cell count data to issue and lift recreational advisories has been a concern for many. Specifically, there is no standard method for performing cell counts that provides assurance that cells are counted consistently across laboratories. Current research with concurrence from the Environmental Protection Agency (EPA) points out that there is uncertainty about the relationship between cell counts and the level of toxins produced. Other research (Manganelli et al., 2010) suggests that cell count alone is not a good predictor of human health risk. In fact, the State of Washington's Department of Ecology uses only cyanotoxin testing data as a basis for public health advisories.

Between August 21 and August 30, 2009, four dogs died of acute anatoxin-a poisoning shortly after drinking water from Elk Creek and the Umpqua River near the confluence of these two streams at Elkton, Oregon.

Water samples collected from the area on September 1, 2009 had no detectable toxigenic cyanobacteria. However, other samples collected from the same areas on the same day revealed detectable levels of anatoxin-a (0.5 µg/L). Microcystin was measured at an average concentration of 15 µg/L (1.5 times above the advisory threshold at the time of 10 µg/L). There was no visible bloom or scum reported in that area of the creek when these fatalities occurred. This case demonstrates that lethal concentrations of cyanotoxins can be present in the absence of detectable toxigenic cyanobacterial cells. Due to the uncertainty associated with cell densities, level of toxin production and exposure to people and pets, OHA has removed cell count data from the advisory issuing and lifting protocol.

Appendix B: Toxigenic cyanobacteria and related cyanotoxin information

A variety of genera of cyanobacteria are capable of producing toxins that are harmful to people, pets and wildlife (Chorus and Bartram, 1999). The most common toxigenic genera observed during CyanoHABs in Oregon are *Microcystis* and *Dolichospermum.*

Microcystis can produce microcystin (liver toxin) and anatoxin-a (neurotoxin). *Dolichospermum*, in addition to producing microcystin and anatoxin-a, can also produce cylindrospermopsin (liver toxin) and saxitoxin (neurotoxin). A complete listing of toxigenic cyanobacteria considered when issuing health advisories in Oregon is presented in Table B-1 on page 9.

Table B-1. Toxigenic cyanobacteria (data derived from evidence of toxin production (Chorus and Bartram, 1999; Carey et al., 2007; Funari and Testai, 2008; Voloshko et al., 2008))

	Hepatotoxin (liver toxins)			*Neurotoxins*	
	Microcystin	Nodularin	Cylindro-spermopsin	Anatoxin-a	Saxitoxin
Anabaenopsis	+				
Aphanizomenon	+		+	+	+
Arthrospira	+				
Cyanobium	+				
Cylindrospermopsis			+		+
Dolichospermum	+		+	+	+
Gloeotrichia	+				
Hapalosiphon	+				
Limnothrix	+				
Lyngba					+
Microcystis	+			+	
Nodularia		+			
Nostoc	+				
Oscillatoria	+			+	
Phormidium	+			+	
Planktothrix	+			+	+
Raphidiopsis			+	+	
Schizothrix					
Synechocystis	+				
Umezakia			+		
Woronichinia	+			+	

Note: Table B-1 is at the genus level. Not all species of a given genus produce all the toxins listed for that genus. Once the species involved in a specific bloom have been identified, OHA recommends that water body mangers contact OHA to determine exactly which toxins could be involved. Taxonomy for many types of cyanobacteria is currently being revised. This guidance reflects taxonomy as of 1/2017.

The primary cyanotoxins of concern in Oregon are microcystin, anatoxin and cylindrospermopsin because they have been the toxins most frequently tested and detected. However, small amounts of saxitoxin have also been detected in Oregon. OHA recommends testing for the cyanotoxins listed in Table B-1 to issue and lift advisories when genera that produce those toxins are present. Health advisories are not issued solely for algal production of lipopolysaccharides (LPS) as these compounds are produced by most algal species, and exposure to LPS compounds typically produce mild, self-limiting rashes in sensitive people.

Microcystin

Background

Microcystins are the most commonly detected cyanotoxin in the world. Cyanobacteria known to produce Microcystins include *Microcystis, Planktothrix, Oscillatoria, Nostoc, Dolichospermum, Anabaenopsis* and *Hapalosiphon*. Microcystins are cyclic heptapeptides with about 60 known structural variants (Rinehart et al., 1994). These variations have significant influence on the toxicity and physio-chemical properties of the toxin. The most studied variant is microcystin-LR.

The mechanism of toxicity of microcystins is the inhibition of protein phosphatases which can cause internal hemorrhaging of the liver. While the inhibition of protein phosphatases may be generally cytotoxic, the microcystins primarily target liver cells since they enter cells through a bile acid carrier most abundant on liver cells.

Exposure to microcystin has the potential to cause acute and chronic injury, depending on dose and duration of exposure. Sub-acute damage to the liver is likely to go unnoticed up to levels that are near severe acute damage (Chorus et al., 2000). Two aspects of chronic damage include progressive injury to the liver and tumor-promoting capacity. Microcystins alone have not been classified as carcinogenic. However, microcystins are considered to be tumor promoters based on studies in mice (Falconer and Buckley, 1989).

Most of the mammalian poisonings from the ingestion of microcystin have involved livestock. Symptoms reported from cattle that were exposed to *Microcystis aeruginosa* include generalized weakness, hyperthermia, anorexia, diarrhea, pale mucous membranes, mental derangement, muscle tremors, coma and death within a few hours to days (Short and Edwards, 1990). Symptoms reported from British military recruits exposed to a bloom of *M. aeruginosa* during an exercise included abdominal pain, vomiting, diarrhea, sore throat, blistering of the mouth and pneumonia (Turner et al., 1990).

OHA used a 28-day rat study (Heinze, 1999) as the critical study for determining a tolerable daily intake (TDI). In this study, researchers treated rats with purified microcystin LR in drinking water for 28 days then measured several endpoints. The Heinze study identified a lowest observable adverse effect level (LOAEL) of 50 µg/kg-day.

Provisional Tolerable Daily Intake

HABS used the LOAEL identified in the Heinze study (Heinze, 1999) described above (50 µg/kg-day) to derive a provisional TDI of 0.05 µg/kg-day as follows:

$$\mathrm{TDI} = \frac{\mathrm{LOAEL}}{\mathrm{UF}}$$

Where:

TDI = Tolerable Daily Intake (0.05 µg/kg-day)
LOAEL = Lowest Observable Adverse Effect Level (50 µg/kg-day)
UF = Uncertainty Factors (1,000 Total = 10 for LOAEL to NOAEL adjustment * 10 for interspecies variability * 10 for individual variability)

This TDI is intended for use with acute or short-term exposure scenarios and may not be protective for chronic or long-term exposures. This recommended TDI should be considered provisional and

will be updated to conform to federal guidelines or standards when they are issued, or whenever additional toxicological information becomes available.

Additional support for this TDI: The EPA has used this same TDI as their reference dose (RfD) for microcystins based on currently available research.

Provisional Recreational Use Value

OHA used the TDI of 0.05 µg/kg-day above to derive a provisional **recreational use value of 8 µg/L for microcystin**:

$$\text{Recreational Use Value} = \frac{\text{TDI} \times \text{RSC} \times \text{BW}}{\text{IR}}$$

Where:

TDI = Tolerable Daily Intake (0.05 µg/kg-day)
RSC = 1.0 (U.S. EPA 2000a; Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin 2019)
BW = Mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
IR = Recreational water incidental ingestion rate for children (0.21 L/d) at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997; Dufour et al. 2017)

The TDI was developed by OHA based on oral administration of microcystin-LR via drinking water in rats and effects on the liver (Heinze, 1999).

The mean body weight (BW) of 31.8 kg was used to represent a child between the age of 6 and 11 years. An incidental ingestion rate (IR) was based on EPA guidance for incidental ingestion of recreational water for children at the 90th percentile.

The RUV for microcystin was the result of new research on exposure factors provided by the EPA, specifically affecting body weight, ingestion rate and relative source contribution factors.

Summary

OHA adopted a health-based RUV for microcystin:

- Tolerable Daily Intake: 0.05 µg/kg-day
- **Recreational Use Value: 8 µg/L**

The primary limitation in the database relates to chronic toxicity. Because OHA only intends to apply these RUVs in acute or short-term exposure scenarios, there is no extrapolation from acute to chronic toxicity. Therefore, OHA considered the uncertainty factor for database limitations to be unnecessary.

Anatoxin-a

Background

OHA reviewed available literature on the toxicology of anatoxin-a (Astrachan et al., 1980; Astrachan and Archer, 1981; Fawell and James, 1994; Chorus and Bartram, 1999; Fawell et al., 1999b; Duy et al., 2000; Rogers et al., 2005; Codd et al., 2005; Falconer and Humpage, 2005; van Apeldoorn et al., 2007; Burch, 2008; Pegram et al., 2008) as well as accepted and proposed threshold values used in other governmental jurisdictions (New Zealand Ministry of Health, 2002; USEPA, 2006; Washington Department of Health, 2008).

OHA selected a study conducted by Fawell et al. (Fawell and James, 1994; Fawell et al., 1999b) as the critical study for derivation of a TDI. In this study, groups of 10 male and 10 female mice were orally treated with anatoxin-a every day for 28 days at 4 doses (0, 100, 500, and 2,500 µg/kg-day). The mice were observed for health effects over the course of the experiment and many health-related endpoints and physiological parameters were measured (Fawell and James, 1994; Fawell et al., 1999b).

Three animals died during the study. One of the deaths was not related to treatment but rather resulted from animals fighting in their cages. Two of the deaths, one at 500 µg/kg-day and one at 2,500 µg/kg-day, could have been related to treatment. None of the surviving animals had any observable adverse health effects. Therefore, OHA selected 100 µg/kg-day as the no observable adverse effect level (NOAEL).

Provisional Tolerable Daily Intake

OHA used the NOAEL identified in the Fawell et.al. study (Fawell and James, 1994; Fawell et al., 1999b) described above (100 µg/kg-day) to derive a provisional TDI of 0.1 µg/kg-day as follows:

$$\mathrm{TDI} = \frac{\mathrm{NOAEL}}{\mathrm{UF}}$$

Where:

TDI = Tolerable Daily Intake (0.1 µg/kg-day)
NOAEL = No Observable Adverse Effect Level (100 µg/kg-day)
UF = Uncertainty Factors (1,000 Total = 10 for interspecies variability * 10 for Individual variability * 10 for limitations in the database)

This TDI is intended only for use in acute or short-term exposure scenarios because the toxicity study upon which this TDI is based was short-term. Because most exposures in Oregon are acute or short-term, an acute or short-term TDI is the most useful.

OHA applied a total uncertainty factor of 1,000. This number is a composite of 3 types of uncertainty about this TDI. First, the critical study was conducted in mice, which may have physiological differences in the way they absorb, distribute, metabolize and excrete anatoxin-a relative to humans. Mice may also be more or less sensitive to anatoxin-a toxicity than humans. Therefore, an uncertainty factor of 10 was applied to account for these potential interspecies differences in sensitivity to anatoxin-a.

Second, humans could have considerable individual variability in their sensitivity to anatoxin-a. For example, a child may be more sensitive than an adult or people with certain genetic traits may be

more sensitive than the general population. Therefore, another uncertainty factor of 10 was applied to account for this individual variability. Finally, OHA applied an additional uncertainty factor of 10 due to limitations in the database. Very few applicable studies have been conducted to identify dose-response relationships to anatoxin-a administered orally. Therefore, this uncertainty factor accounts for the possibility that additional studies in the future may reveal that anatoxin-a is more toxic than has been suggested in the currently available literature.

This recommended TDI should be considered provisional because of the paucity of toxicity data. OHA will update this TDI when more toxicity information becomes available.

Additional studies supporting this TDI: OHA only identified two primary studies that employed oral administration of anatoxin-a: the Fawell, et.al. study selected as the critical study (Fawell and James, 1994; Fawell et al., 1999b), and an older study conducted by Astrachan, et al. (Astrachan et al., 1980; Astrachan and Archer, 1981).

Independent reviews (Duy et al., 2000; Codd et al., 2005) of this Astrachan, et al. study have derived a TDI of 0.51 µg/kg-day, a value similar within a factor of 5 to the TDI selected (0.1 µg/kg-day). California's Environmental Protection Agency (CalEPA) has proposed an oral reference dose of 0.5 µg/kg-day (CalEPA, 2012), a value similar within a factor of 5 to the TDI selected here.

Other toxicity studies (Rogers et al., 2005) have been conducted using non-oral (mainly intraperitoneal injection) routes of exposure. Because human exposures to anatoxin-a in Oregon is expected to be primarily through ingestion, either in drinking water or accidental ingestion of surface water while recreating, OHA only considered studies using the oral route of exposure.

Provisional Recreational Use Value

OHA used the TDI of 0.1 µg/kg-day above to derive a provisional **recreational use value of 15 µg/L for anatoxin-a:**

$$\text{Recreational Use Value} = \frac{\text{TDI} \times \text{RSC} \times \text{BW}}{\text{IR}}$$

Where:

TDI = Tolerable Daily Intake (0.1 µg/kg-day)
RSC = 1.0 (U.S. EPA 2000a; Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin 2019)
BW = Mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
IR = Recreational water incidental ingestion rate for children (0.21 L/d) at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997; Dufour et al. 2017)

The RUV for anatoxin-a was the result of new research on exposure factors provided by the Environmental Protection Agency (EPA) for microcystin and cylindrospermopsin, specifically affecting body weight, ingestion rate and relative source contribution factors. These same factors were used to calculate the RUV for anatoxin-a.

This RUV is based on a provisional TDI. Therefore, this value should also be considered provisional and subject to change should the provisional TDI be updated to accommodate new scientific information.

Summary

OHA adopted health-based RUVs for anatoxin-a:

- Tolerable Daily Intake: 0.1 µg/kg-day
- **Recreational Use Value: 15 µg/L**

As noted above, very few studies have been done to quantify the oral dose-response to anatoxin-a. Therefore, these RUVs should be viewed as provisional and subject to revisions pending further research relevant to anatoxin-a toxicity.

Saxitoxins

Background

Saxitoxins (STXs) are a family of biological toxins associated with paralytic shellfish poisoning (PSP). This family includes saxitoxin (STX), neosaxitoxin (neoSTX), gonyautoxins, (GTX), C-toxins (C), 11-hydroxy-STX and decarbamoylsaxitoxins (dcSTXs)(van Apeldoorn et al., 2007). Because individual STXs vary in their toxicity, the European Food Safety Authority (EFSA) developed toxic equivalency factors (TEFs), based on toxicity in mice, so individual toxin concentrations can be considered relative to the toxicity of STX (EFSA, 2009). The proposed TEFs are: STX = 1, NeoSTX = 1, GTX1 = 1, GTX2 = 0.4, GTX3 = 0.6, GTX4 = 0.7, GTX5 = 0.1, GTX6 = 0.1, C2 = 0.1, C4 = 0.1, dc-STX = 1, dc-NeoSTX = 0.4, dc-GTX2 = 0.2, GTX3 = 0.4, and 11-hydroxy-STX = 0.3 (EFSA, 2009).

OHA adopted these TEFs as the method for reporting STX-equivalents (STX-eq) results for public health analysis in Oregon. Most labs report total saxitoxins, which is also acceptable. Previously few waterbody managers tested for this cyanotoxin because it was considered an insignificant threat in the Northwest. However from 2009 to 2011, 4 of 30 Washington State lakes sampled tested positive for saxitoxin (Hardy and Farrer, 2011).

Given the documented presence of saxitoxin in Washington, it was important to determine whether this cyanotoxin was also present in Oregon. Since development of RUVs for saxitoxins in recreational waters by OHA, this toxin has been detected in Oregon waters. OHA asks water body managers to provide saxitoxin data when a waterbody contains taxa of cyanobacteria associated with this toxin.

EFSA established an acute RfD for STX-eq of 0.5 µg STX-eq/kg-day (EFSA, 2009). This acute RfD is based on available intoxication reports in humans across the European population. This acute RfD represents an estimated NOAEL.

Provisional Tolerable Daily Intake

OHA used the RfD/NOAEL described above (0.5 µg/kg-day) to derive a provisional TDI of 0.05 µg/kg-day as follows:

$$\mathrm{TDI} = \frac{\mathrm{NOAEL}}{\mathrm{UF}}$$

Where:

TDI = Tolerable Daily Intake (0.05 µg/kg-day)
NOAEL = No Observable Adverse Effect Level (0.5 µg/kg-day)
UF = Uncertainty Factors (10 for limitations in the database).

This TDI is based on an acute toxicity study, so it is only applicable to acute or short-term exposure scenarios. OHA applied a total uncertainty factor of 10 for database limitations[1]. This is the only study of its kind for saxitoxin and additional studies may find a lower RfD.

For humans, no uncertainty factor for interspecies variability was needed since the data were from human illnesses. OHA also did not apply an uncertainty factor for individual variability since the EFSA study covered the general population which included sensitive individuals.

Provisional Recreational Use Value

OHA used the TDI of 0.05 µg/kg-day to derive a provisional **recreational use value of 8 µg/L for SXT-eq:**

$$\text{Recreational Use Value} = \frac{\text{TDI} \times \text{RSC} \times \text{BW}}{\text{IR}}$$

Where:

TDI= Acute oral reference dose (0.05 µg STX-eq/kg-day)
RSC = 1.0 (U.S. EPA 2000a; Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin 2019)
BW = Mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
IR = Recreational water incidental ingestion rate for children (0.21 L/d) at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997; Dufour et al. 2017)

The RUV for saxitoxin was the result of new research on exposure factors provided by the Environmental Protection Agency (EPA) for microcystin and cylindrospermopsin, specifically affecting body weight, ingestion rate and relative source contribution factors. These same factors were used to calculate the RUV for saxitoxin.

OHA applies this SXT-eq RUV to total saxitoxin results. This provisional RUV is based on EFSA's acute RfD. This value is subject to change should additional toxicological information become available in the future.

Summary

OHA adopted health-based RUVs for saxitoxin:

- Tolerable Daily Intake: 0.05 µg/kg-day
- **Recreational Use Value: 8 µg STX-eq/L**

As noted above, this value should be viewed as provisional and subject to revisions pending further research relevant to STX toxicity.

[1]OHA did not originally apply the uncertainty factor for database limitations to the TDI for saxitoxins. Application of this uncertainty factor dropped OHA's previous TDI and all RUVs based on that TDI (recreational water RUVs and drinking water GVs) by a factor of 10. OHA applied the database limitation uncertainty factor in this revision in keeping with the Ohio EPA, which first applied this uncertainty factor in 2014.

Cylindrospermopsin

Background

Previously, few waterbody managers tested for this cyanotoxin because it had been considered an insignificant threat in the Northwest. However, in 2011, a water body in Washington tested positive for cylindrospermopsin (Hardy and Farrer, 2011). Since 2011, cylindrospermopsin has been detected in Oregon above the RUV established by OHA. Given the documented presence of cylindrospermopsin in Washington and Oregon, OHA asks waterbody managers to provide cylindrospermopsin data when a waterbody contains taxa of cyanobacteria associated with this toxin.

Tolerable Daily Intake

To develop a TDI for cylindrospermopsin, OHA used the same study by Humpage et. al., 2003 that the EPA selected as the critical study in development of their 10-day Health Advisory for cylindrospermopsin. This 11-week study used male Swiss albino mice in which groups of mice were dosed with 0, 30, 60, 120, or 240 µg/kg-day (10 mice per dose group) of purified cylindrospermopsin by daily gavage. Authors monitored food and water consumption and body weights throughout the study. At nine weeks, authors conducted clinical exams with a focus on physiological and behavioral signs of toxicity. Near the end of the study an extensive panel of parameters was measured in serum and urine along with hematological endpoints. No deaths were reported in the study. Upon necropsy, organs were weighed, and all tissues were examined histologically. The most sensitive endpoint observed was kidney weight, which increased in a dose-dependent manner starting at 60 µg/kg-day. The EPA selected 60 µg/kg-day from this study as the LOAEL and 30 µg/kg-day as the NOAEL [23].

Consistent with EPA's Health Advisory methodology, OHA applied a total uncertainty factor of 300 to the NOAEL of 30 µg/kg-day. The total UF of 300 was a composite of an UF of 10 for interspecies variability, 10 for individual variability, and 3[2] for database limitations. OHA used the NOAEL of 30 µg/kg-day to derive a provisional TDI of 0.1 µg/kg-day as follows:

$$TDI = \frac{NOAEL}{UF}$$

Where:

TDI = Tolerable Daily Intake (0.1 µg/kg-day)
NOAEL = No Observable Adverse Effect Level (30 µg/kg-day)
UF = Uncertainty Factors (300).

The EPA has also adopted this same TDI as their reference dose (RfD) for Cylindrospermopsin.

[2] The previous assessment of cylindrospermopsin included a database limitation factor of 10. An uncertainty factor of 3 was used in the current 10-day Health Advisory issued by the EPA's Office of Water on June 17, 2015. To be consistent with EPA guidance, OHA adopted this uncertainty factor which resulted in an increase in the TDI from the previous value by an approximate factor of 3.

Provisional Recreational Use Value

OHA used the TDI of 0.1 µg/kg-day above to derive a provisional **recreational use value of 15 µg/L for cylindrospermopsin:**

$$\text{Recreational Use Value} = \frac{\text{TDI} \times \text{RSC} \times \text{BW}}{\text{IR}}$$

Where:

TDI = Tolerable Daily Intake (0.1 µg/kg-day)
RSC = 1.0 (U.S. EPA 2000a; Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin 2019)
BW = Mean body weight of children 6 to < 11 years (31.8 kg) (U.S. EPA 2011)
IR = Recreational water incidental ingestion rate for children (0.21 L/d) at approximately the 90th percentile (U.S. EPA 2011; U.S. EPA 1997; Dufour et al. 2017)

The mean body weight (BW) of 31.8 kg was used to represent a child between the age of 6 and 11 years. An incidental ingestion rate (IR) was based on EPA guidance for incidental ingestion of recreational water for children at the 90th percentile, and from a recent study by Dufour et al. (2017).

The RUV for cylindrospermopsin was the result of new research on exposure factors provided by the EPA, specifically affecting body weight, ingestion rate and relative source contribution factors.

Summary

OHA adopted health-based RUVs for cylindrospermopsin:

- Tolerable Daily Intake: 0.1 µg/kg-day
- **Recreational Use Value: 15 µg/L**

OHA adopted a RUV of 15 µg/L for cylindrospermopsin based on EPA criteria. As noted above, this value should be viewed as provisional and subject to revisions pending further research relevant to cylindrospermopsin toxicity.

Appendix C: Exposure pathways

The primary pathway for exposure to cyanotoxins is ingestion of water. Dermal effects are possible from the lipopolysaccharides found on cell surfaces, however, cyanotoxins are not likely to cross the skin barrier and enter the bloodstream. Inhalation and aspiration of toxin is possible, especially through activities where the toxin is aerosolized, such as water skiing or splashing.

Ingestion of water can occur through both incidental and intentional ingestion. The risk of incidental ingestion is particularly high for children playing in near-shore areas where scum tends to accumulate. Exposure levels can be broadly defined as high, moderate and low based on recreational activity (Table C-1).

Table C-1. Level of recreational activity (modified from Queensland Health, 2001)

Level of Exposure	*Recreational Activity*
High	Swimming, diving, water skiing
Moderate	Canoeing, sailing, rowing
Low to none	Fishing, pleasure cruising, picnicking, hiking

Two possible scenarios for human intentional ingestion of recreational water should be considered. One is lake water used for drinking or cooking purposes by campers and hikers. Boiling, or use of camping style equipment for filtering or treating affected water will not make it potable, and in fact, can make the toxins more concentrated. The second risk for exposure occurs when people draw in-home water directly from a lake or river. Many private treatment systems have not been proven effective in removing cyanotoxins. This exposure information is addressed in all advisory news releases, educational materials and signs.

Note: There is currently one manufacturer of in-home filtering equipment that certifies the reduction or elimination of microcystin in affected water. More information about this filtration system can be found through NSF Contaminant Reduction Claims Guide. http://www.nsf.org/consumer-resources/water-quality/water-filters-testing-treatment/contaminant-reduction-claims-guide.

Public Drinking Water Systems

Drinking water is another exposure pathway of concern for cyanotoxins. Occasionally, CyanoHABs occur in recreational waters used as drinking water sources. OHA's Drinking Water Program has adopted the acute toxicity values for cyanotoxins in drinking water established by the EPA (Table C-2). Drinking water containing cyanotoxins above the acute values in Table C-2 could cause immediate harm to public health. Although these are not enforceable Maximum Contaminant Levels (MCLs), OHA recommends that public water systems use them as "Do Not Drink" thresholds.

Table C-2. Acute or short-term drinking water cyanotoxin toxicity values (µg/L)

Drinking Water Guidance Value:	*Microcystin*	*Cylindrospermopsin*	*Saxitoxin*	*Anatoxin-a*
Adults	1.6	3	1.6*	3
Ages 5 years and younger	0.3	0.7	0.3	0.7

*OHA's previous drinking water guidance value for saxitoxin was 3 µg/L and was based on guidance used in other countries and not a TDI. This new drinking water value is based on the TDI established in Appendix B.

Note: *Rounding conventions are consistent with EPA's 10-day Health Advisories*

For information regarding these guideline values, contact OHA at 971-673-0440 or HAB.health@state .or.us. For more guidance specific to drinking water system operators, visit: http://public.health.oregon.gov/HealthyEnvironments/DrinkingWater/Operations/Treatment/Pages/algae.aspx.

Table C-3 lists the exposure factors used to calculate drinking water Guideline Values (GVs) using the TDIs established in Appendix B. The equation used to calculate drinking water GVs is identical to the equation used to calculate RUVs in Appendix B.

Table C-3. Exposure factors used to calculate drinking water GVs

Parameter	Adults	Children 5 and younger
Body Weight	80 kilograms	---
Intake Rate	2.5 liters	---
Body Weight-Normalized Intake Rate	---	0.15 liters/kilogram-body weight per day

Note: OHA adopted EPA's exposure factors used in their derivation of 10-day Health Advisories for microcystin and cylindrospermopsin and applied them to the TDIs OHA derived for anatoxin-a and saxitoxins as well. Although drinking water treatment facilities are only required to sample for microcystin and cylindrospermopsin, the levels for saxitoxin and anatoxin-a can be used for informational purposes.

Fish Consumption

At this time, there is insufficient information to determine the risk of consuming fish caught in waters with a CyanoHAB. Studies have shown that toxins mainly accumulate in the liver and viscera of fish, and small amounts of microcystin has been detected in the fillet (Vasconcelos, 1999; de Magalhaes et al., 2001; Kann, 2008; Washington Department of Ecology, 2010; Kann et al., 2011). At a minimum, organs and skin should be removed and discarded, and fillets rinsed with clean water prior to cooking or freezing fillets. Caution should be taken with shellfish as cyanotoxins have been shown to accumulate in edible tissue (Vasconcelos, 1999).

Risk to Animals

Animals are extremely sensitive to cyanotoxins when present and can become very ill or potentially die due to exposure at very low levels. The primary route of exposure to these toxins is through ingestion. Ingestion occurs when pets and wildlife drink water from a cyanobacteria-filled lake or pond, lick their fur after swimming, or eat dried cells that accumulate along the shoreline.

Because dogs are cyanotoxin sensitive animals and dog deaths have been confirmed due to CyanoHABs, OHA developed dog-specific RUVs for cyanotoxins in recreational water (Table C-4).

Table C-4. Dog-specific RUVs for cyanotoxins (µg/L)

Dog RUV:	*Anatoxin-a*	*Cylindrospermopsin*	*Microcystin*	*Saxitoxin*
	0.4	0.4	0.2	0.02

Note: All dog-specific RUVs have been changed in this revision because California EPA's estimate of the amount of water an exercising dog consumes per kilogram body weight was updated in 2012 (from 0.168 to

0.255 L/kg-day). Current dog-specific RUVs are now consistent with the California EPA update. The dog-specific value for saxitoxins was further modified by application of an uncertainty factor to the dog-specific TDI for interspecies differences in sensitivity between humans (the species in the critical study) and dogs.

OHA does not use these dog-specific RUVs as the basis for public health advisories. Rather, they are offered as a resource to veterinarians and veterinary associations to use as appropriate, when treating dogs believed to have been exposed to cyanotoxins. OHA will use these values and potential exposure scenarios in discussions with individual veterinarians or pet owners, to educate them on the vulnerability of pets to cyanotoxin exposure. Contact OHA for details about the origin of these dog-specific values.

Note: Pet owners should be aware that the RUVs for dogs is below the GVs for drinking water affected by cyanotoxins. Because of this, OHA recommends owners supply their pets with bottled water or water from alternative sources when a drinking water advisory is in place.

Appendix D: References

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Attachment 3

Cutaneous Hypersensitivity Reactions to Freshwater Cyanobacteria – Human Volunteer Studies
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Cutaneous hypersensitivity reactions to freshwater cyanobacteria – human volunteer studies

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Abstract

Background: Pruritic skin rashes associated with exposure to freshwater cyanobacteria are infrequently reported in the medical and scientific literature, mostly as anecdotal and case reports. Diagnostic dermatological investigations in humans are also infrequently described. We sought to conduct a pilot volunteer study to explore the potential for cyanobacteria to elicit hypersensitivity reactions.

Methods: A consecutive series of adult patients presenting for diagnostic skin patch testing at a hospital outpatient clinic were invited to participate. A convenience sample of volunteers matched for age and sex was also enrolled. Patches containing aqueous suspensions of various cyanobacteria at three concentrations were applied for 48 hours; dermatological assessment was made 48 hours and 96 hours after application.

Results: 20 outpatients and 19 reference subjects were recruited into the study. A single outpatient produced unequivocal reactions to several cyanobacteria suspensions; this subject was also the only one of the outpatient group with a diagnosis of atopic dermatitis. No subjects in the reference group developed clinically detectable skin reactions to cyanobacteria.

Conclusion: This preliminary clinical study demonstrates that hypersensitivity reactions to cyanobacteria appear to be infrequent in both the general and dermatological outpatient populations. As cyanobacteria are widely distributed in aquatic environments, a better appreciation of risk factors, particularly with respect to allergic predisposition, may help to refine health advice given to people engaging in recreational activities where nuisance cyanobacteria are a problem.

Background

Cyanobacteria, commonly but erroneously known as blue-green algae, are common inhabitants of freshwater lakes and reservoirs throughout the world. Under favour-

able conditions certain cyanobacteria can dominate the phytoplankton within a waterbody and undergo mass developments, known as blooms. Public health concerns arise because many nuisance cyanobacteria can produce potent toxins. Anecdotal and case reports have documented skin rashes, often described as intensely pruritic, associated with contact exposure to cyanobacteria. While there are relatively few references in the scientific and medical literature since these reports began in 1949, under-diagnosis of cyanobacteria-associated illness was suggested by Schwimmer & Schwimmer [1] in 1968, a suspicion that probably holds today. Most reports of cyanobacteria-associated skin eruptions describe recreational or occupational exposure [2], however there are anecdotal reports of skin rashes related to water treatment failures and subsequent presence of cyanobacterial products in reticulated supplies. In these instances, skin rashes were reported after showering or bathing [3,4]. "Several" people experienced acute dermatitis, as well as gastrointestinal symptoms, after drinking water from a riverine source affected by a cyanobacteria bloom in Portugal [5].

Skin patch testing is a routine diagnostic procedure in dermatology clinics worldwide, and testing with cyanobacterial preparations was first reported in the USA in 1953 to investigate a water contact-related seasonal dermatitis in a girl aged six years. Strong positive reactions to various extracts of an *Anabaena* sp. dominant bloom sample were observed on the child but none of 25 healthy control subjects [6].

In a study of volunteers to investigate irritant reactions, Pilotto *et al* [7] reported that 20–24% of subjects reacted to cyanobacterial test patches, and 23% of subjects responded to negative control patches. After excluding subjects who responded to the negative controls, 11–15% of subjects responded to cyanobacteria. No dose-response relationships were reported.

Anecdotal and case reports in the medical and scientific literature do not provide convincing descriptions of mass outbreaks of cutaneous symptoms associated with recreational or occupational exposure to planktonic cyanobacteria. Rather, the picture is of isolated events affecting individuals or small numbers of people [2]. An epidemiological study to investigate the occurrence of acute symptoms did not find a statistically significant difference in the reporting of cutaneous symptoms across groups exposed to different levels of planktonic cyanobacteria in recreational waters. The small number of subjects that reported skin ailments after bathing in cyanobacteria-affected waters mostly rated the severity of symptoms as mild [8]. Taken together, these findings suggest that nuisance planktonic cyanobacteria are not commonly present at irritant concentrations in inland recreational waters, unlike the marine filamentous cyanobacterium *Lyngbya majuscula*, which is known to produce dermally-active toxins and has been linked to mass outbreaks of acute dermatitis involving hundreds of individuals, with high proportions of exposed individuals being affected [9].

The purpose of this study was to assess the propensity for a range of cyanobacterial suspensions to induce cutaneous irritant and hypersensitivity reactions in dermatology outpatients and a reference group of volunteers matched for age and sex. We wished to determine whether threshold doses that induce reactions in the reference group, if indeed such reactions occur in this group, are lower in individuals with an active history of cutaneous symptoms. Irritant and hypersensitivity reactions would be determined both qualitatively and quantitatively, and the cyanobacteria would be characterised in terms of species (or genera if speciation were not possible), doses to be applied to the skin, and the presence or absence of known toxins.

Methods

Study participants, patch application and reading

A consecutive series of adults aged 18 to 65 years presenting for diagnostic skin patch testing at the Royal Brisbane and Women's Hospital dermatology outpatient clinic between March 2002 and November 2003 was invited to participate in the study – provided they met study inclusion criteria – until 20 were recruited. A convenience sample of volunteers was recruited via notices posted at three university sites and by word of mouth for the reference group. Patients and reference subjects were matched by sex and, where possible, by age using 5 year age bands. Routine exclusion criteria for elective patch testing applied to this study: persons with infectious dermatoses, widespread acne, traumatic lesion or excess hair on their back. Pregnant women were also excluded.

Study subjects were asked to complete a simple questionnaire that requested basic demographic details (age, sex), history of allergic illness (asthma, hay fever, eczema, urticaria), relevant medications and a description of any freshwater-related dermatoses [[10] (Appendix 4)].

The skin patch testing procedure uses a series of shallow aluminium chambers (Finn chambers), 8 mm internal diameter, 0.5 mm depth, into which test materials are placed, either impregnated onto discs of filter paper or mixed in petrolatum [11]. Test material is placed in each chamber, and the Finn chamber strips are fixed on the skin with non occlusive, non-allergenic and non-irritant adhesive tape. For this study a clinic nurse prepared the skin of each subject's back with acetone, and patches were applied to the skin. Study subjects were instructed to keep

Table 1: Patch testing interpretation key

+/-	Uncertain reaction: faint macular erythema only
+	*Weak* (nonvesicular) positive reaction; erythema, infiltration, possibly papules
++	*Strong* (vesicular) positive reaction; erythema, infiltration, papules, vesicles
+++	*Extreme* positive reaction; bullous reaction
-	*Negative* reaction
IR	Irritant reaction of different types

Adapted from Rietschel & Fowler [16 (p. 24)] (interpretation key of the International Contact Dermatitis Research Group).

their back dry, i.e. bathe but not shower, and to refrain from sport or vigorous activity that might lead to frank perspiration, with resultant separation of Finn chamber strips from the skin. Patches were then removed after 48 hours. The clinic nurse marked the position of each Finn chamber with a permanent marker pen; after allowing adhesive tape-related erythema to subside, patch test sites were read by a dermatologist after 48 and approximately 96 hours. Patch sites were scored according to the key in Table 1.

Dermatology clinic workers were blinded to the identity of test materials (patch series columns) but not to test concentrations (patch series rows) because we thought that identification of concentration-dependent (i.e. dose-response) reactions to any particular test suspension series would assist in the differentiation of irritant and hypersensitivity responses. Clinic workers were not blinded to the status of study subjects as either patients or non-patients.

Ethical approvals for this study and amendments were granted by the Royal Brisbane Hospital Health Service District's Human Research Ethics Committee, protocol number 2001/151, and the University of Queensland's Medical Research Ethics Committee, clearance number 2002000099.

Patch test materials

Six cyanobacterial suspensions, two cyanobacterial lipopolysaccharide extracts and one eukaryotic algal suspension were tested, each at three concentrations. Sodium dodecyl sulfate was used as a positive irritant control. The test materials and measured cyanotoxin concentrations are listed in Table 2.

Culturing of cyanobacterial isolates; preparation of stock suspensions

Cyanobacteria isolates were non-axenic laboratory cultures grown in sterile inorganic media in an illuminated growth chamber at 28°C with a 14:10 light/dark cycle. Culture vessels were aerated with aquarium pumps and

Table 2: Suspensions and extracts applied to patch test wells

Test material or species	Patch series type	Strain	Source	Cyanotoxin (concentration in 0.25% w/v lyophilised cyanobacteria patch preparation)
Sodium dodecyl sulfate (SDS, aka sodium lauryl sulfate)	Positive irritant control		Sigma-Aldrich P/L	
Cylindrospermopsis raciborskii	Cyanobacterial cell suspension	AWT 205 Non-axenic	Australian Water Technologies culture collection Sydney, Australia	Cylindrospermopsin (2.0 mg/L)
C. raciborskii	Cyanobacterial LPS extract	AWT 205 Non-axenic	Australian Water Technologies culture collection Sydney, Australia	
Microcystis aeruginosa *C. raciborskii* *Aphanizomenon* sp.	Cyanobacterial cell suspension		Field sample, North Pine Dam (South-east Queensland, Australia)	Microcystins (200 μg/L total microcystins expressed as MC-LR); cylindrospermopsin (6.4 μg/L)
M. aeruginosa	Cyanobacterial cell suspension		Field sample, Lake Coolmunda (Southern Queensland, Australia)	Non-toxic (nil detect for microcystins)
M. aeruginosa	Cyanobacterial LPS extract		Field sample, Lake Coolmunda (Southern Queensland, Australia)	
M. aeruginosa	Cyanobacterial cell suspension	QH/NR/Ma/03 Non-axenic	Queensland Health Scientific Services culture collection, Brisbane, Australia	Microcystins [predominantly microcystin-LR] (1.60 mg/L total microcystins expressed as MC-LR)
Anabaena circinalis	Cyanobacterial cell suspension		Field sample, Lake Coolmunda (Southern Queensland, Australia)	Saxitoxins (19 μg/L total saxitoxins expressed as saxitoxin)
Planktothrix sp.	Cyanobacterial cell suspension	QH/NR/Px/01 Non-axenic	Queensland Health Scientific Services culture collection, Brisbane, Australia	Non-toxic (nil detect for microcystins)
Chlorella vulgaris	Green algal cell suspension	CS-42 Non-axenic	CSIRO collection of living microalgae, Hobart, Australia	

air-stones connected by PVC tubing; air was delivered through 0.45 μm Millipore® filters, and all culture vessels and air delivery components distal to the filter (tubing, weights and air-stones) were sterilised prior to use by steam autoclaving or Sterrad® hydrogen peroxide plasma sterilisation (the latter for heat labile plastics).

M. aeruginosa and *Planktothrix* sp. cultures were grown in 20L batch cultures; *M. aeruginosa* cells were harvested by placing the culture vessel in a darkened cupboard overnight. This caused cells to rise to the surface of the vessel where they were aspirated with a syringe and PVC tubing. *Planktothrix* sp. is a filamentous cyanobacterium, so was easily harvested by plucking it in several continuous sheets from the vessel walls and aeration tubing. *C. raciborskii* was produced by continuous culture adapted from the method of Court *et al* [12] and cells were concentrated by centrifugation in 750 mL centrifuge bottles, then decanting and discarding media. Cells were double-washed by repeat suspension in de-ionised water followed by centrifugation. 250 mL of *C. vulgaris* culture was purchased from CSIRO Hobart, which after double washing yielded sufficient cellular material for this work. Harvested cells were lyophilised, powdered with a domestic coffee grinder and stored at room temperature in air-tight containers.

Stock preparations were made by suspending 25 mg lyophilised cells in 10 mL Milli-Q® filtered water to produce 0.25% w/v suspensions. These were steeped overnight at 4°C. Cell integrity was disrupted by subjecting each suspension to ultrasonic pulsing for 30 seconds, using a Branson Ultrasonics Sonifier 450 instrument. From each 0.25% preparation 1 mL was added to 4 mL Milli-Q® water to produce the 0.05% suspension, and 0.5 mL of that preparation was added to 4.5 mL water for the 0.005% suspension. All suspensions were stored at -20°C.

Lipopolysaccharide (LPS) solutions were prepared from LPS isolated and purified with a hot phenol method and ultracentrifugation, per procedures No. 4: Bacterial lipopolysaccharides – Gram-negative (modified Westphal) and No. 27: Purification of lipopolysaccharide (modified Westphal) ([13], (pp.3-4, 31-2)), from the process described by Westphal & Jann [14]. LPS concentrations were based on the percentage yield from cyanobacterial whole cells they were extracted from:

• *M. aeruginosa* LPS was 0.51% of dry cell weight, so the maximum concentration of LPS for skin patch testing was $(5.1 \times 10^{-3}) \times 0.25\%$ w/v, i.e. 13 ppm. Intermediate and low concentrations were prepared by diluting the 13 ppm concentration as described above to give 3 ppm and 300 ppb concentrations.

• *C. raciborskii* LPS was 1.25% of dry cell weight, so the three concentrations of this LPS were 30 ppm, 6 ppm and 600 ppb.

Sodium dodecyl sulfate was prepared at concentrations of 2.0%, 0.4% and 0.04% (w/v in Milli-Q® water) and stored at -20°C.

Microcystins, saxitoxins and cylindrospermopsin were quantified at Queensland Health Scientific Services, Brisbane. These data are included in Table 2; methodology and instrumentation were as outlined in the accompanying paper by Stewart *et al* [15].

Calculation of cyanotoxin doses applied to skin

Cyanobacterial cell suspensions were applied to filter paper discs that fit into each Finn chamber. A plastic transfer pipette was used to saturate each disc; one or two drops – mostly one drop – are sufficient to saturate the disc. The volume of two transfer pipette drops was measured with an air displacement pipette and found to be 65 μL. Doses were calculated from the maximum concentration (0.25% w/v), then one fifth and one fiftieth of the maximum dose, representing the 0.05% w/v and 0.005% w/v concentrations, were added to estimate the total cutaneous dose for an average 65 kg subject.

Statistical analysis

Comparisons of categorical variables were undertaken using Fisher's exact test. A p-value < 0.05 was used to define statistical significance and all calculations were conducted using SPSS v13.0. Investigation into the incidence of reactions and threshold concentrations of cyanobacteria, adjusted for covariates including reported history of asthma, urticaria or hay fever was planned but not done because only a single subject developed unequivocal reactions to patches containing cyanobacteria.

Results

From the consecutive series of outpatients approached, two declined to participate (one of each sex) and one female who agreed to participate was not included due to an administrative oversight. All outpatients were matched to reference subjects by sex (females: $n = 12$; males: $n = 8$). Matching was also done by age (± 5 years), except for three older outpatient subjects (aged 54, 56 and 62 years).

Responses to the questionnaire enquiry regarding a previous history of allergic illness and acute or chronic skin reactions are summarised in Table 3. Outpatients reported significantly more life-time and recent eczema or dermatitis diagnoses ($p = 0.04$ and $p = 0.01$ respectively), and rash of unknown cause within the last two years ($p = 0.003$) than their reference counterparts.

Table 3: Summary of questionnaire responses: history of cutaneous and allergic illness. *n* (%)

	Outpatients			Reference subjects			
	Yes	No	Not sure	Yes	No	Not sure	p
Eczema or dermatitis							
Ever diagnosed	12(60)	4(20)	1(5)	7(37)	12(63)	0	0.04
Last two years	11(55)	3(15)	3(15)	5(26)	13(68)	0	0.01
Asthma							
Ever diagnosed	6(30)	13(65)	0	8(42)	10(53)	0	0.51
Last two years	5(25)	14(70)	0	5(26)	13(68)	0	1.0
Hay fever							
Ever diagnosed	2(10)	15(75)	1(5)	5(26)	14(74)	0	0.41
Last two years	3(15)	14(70)	1(5)	4(21)	15(79)	0	1.0
Urticaria							
Ever diagnosed	1(5)	17(85)	1(5)	2(11)	16(84)	0	1.0
Last two years	0	17(85)	1(5)	1(5)	16(84)	1(5)	1.0
Rash of unknown cause							
Last two years	10(50)	4(20)	3(15)	3(16)	15(79)	0	0.003
Rash after freshwater recreation	1(5)	16(80)	1(5)	0	15(79)	3(16)	1.0

n = 20 for the outpatient subject group; n = 19 for the reference group. Where sum of row answers (yes/no/not sure) is below the total, shortfall represents unanswered questions.
p-values: Fisher's exact test comparing proportion of "yes" and "no" answers between outpatient and reference subject groups

Skin patch testing – cyanobacterial and algal suspensions

Subjects CO10 and PT05 were removed from consideration of summary statistics given in Table 2. Subject CO10 developed a localised folliculitis over four test series sites – one being the SDS series – so 96-hour readings were uninterpretable. The dermatologist noted a general irritant reaction over the patch area. We were unable to recruit another volunteer in her place, thus the study included 19 reference subjects. Subject PT05 developed "angry back", which is a state of skin hyper-reactivity caused by a strong reaction to one or more patch-test allergens, and is associated with false-positive reactions to other test materials [[16] (pp. 16–17)]; another outpatient subject was recruited to replace this subject in the study.

Table 4 shows results of patch test inspections of the cyanobacterial and algal series. Only one of the outpatient group and none of the reference group showed an unequivocal reaction to cyanobacterial preparations. A weak irritant response to an *A. circinalis* patch was seen in another dermatology outpatient subject, and equivocal responses to various patch materials were seen in four patients and four reference subjects.

Estimated cyanotoxin doses applied to each subject are presented in Table 5. Assuming that two drops of cell suspension were required to saturate each Finn chamber filter disc, and also assuming that the entire volume applied to the discs was in contact with subjects' skin, all doses were well below the mammalian i.p. toxic dose.

Discussion

Patch-testing of cyanobacteria and C. vulgaris

Only one clear response to this skin-patch testing study was seen, from PT19, a male outpatient subject aged 35 years. Interestingly, this subject was also the only one of 20 outpatients with a diagnosis of atopic dermatitis. We did not conduct any statistical analysis of these results, as it is not appropriate to make such comparisons on the basis of a single subject response. This subject developed unequivocal responses to two cyanobacterial isolates, two bloom samples, and probably to *C. vulgaris* as well. There was no evidence of any dose-response effect in the reac-

Table 5: Estimated doses of cyanotoxins by the cutaneous route

Cyanotoxin	Dose per subject	Dose by weight*	Mouse LD_{50}(i.p.)
Cylindrospermopsin	160 ng	2.4 ng/kg	2.1 mg/kg (24 hours); 200 μg/kg (5–6 days) [31]
Microcystins	170 ng	2.6 ng/kg	45–70 μg/kg (most toxic forms) [32 (p. 140)]
Saxitoxins	3.8 ng	58 pg/kg	10–30 μg/kg [32 (p. 140)]

*Dose by weight estimated for a 65 kg individual

Table 4: Cyanobacterial and algal patch series: positive and equivocal patch test results

		Subject								
Test material	**Concentration**	**PT01**	**PTO2**	**PT04**	**PT06**	**PT19**	**CO05**	**CO06**	**CO08**	**CO09**
C. raciborskii	**0.005%**					(++)* (+)**				
AWT 205 cell	**0.05%**					(++)* (+)**				
suspension	**0.25%**					(++)* (+)**				
C. raciborskii	**630 ppb**							(+/-)*		
AWT 205 LPS	**6 ppm**									
extract	**31 ppm**									
M. aeruginosa										
C. raciborskii	**0.005%**					(+)* (+)**				
***Aphanizomenon* sp.**	**0.05%**					(++)* (+)**				
North Pine Dam cell suspension	**0.25%**					(+)* (+)**				
M. aeruginosa	**0.005%**					(+)**				
Lake Coolmunda	**0.05%**					(+)**				
cell suspension	**0.25%**				(+/-)*	(+/-)**				
M. aeruginosa	**260 ppb**							(+/-)*		
Lake Coolmunda	**3 ppm**								(+/-)*	(+/-)**
LPS extract	**13 ppm**				(+/-)*					(+/-)**
M. aeruginosa	**0.005%**						(+/-)*			
QH/NR/Ma/03 cell	**0.05%**									
suspension	**0.25%**		(+/-)*		(+/-)*					
A. circinalis	**0.005%**									
Lake Coolmunda	**0.05%**	(+/- IR)*			(+/-)*					
cell suspension	**0.25%**	(+ IR)*								
***Planktothrix* sp.**	**0.005%**									
QH/NR/Px/01 cell	**0.05%**	(+)*				(+)* (+)**				
suspension	**0.25%**					(+)**				
C. vulgaris	**0.005%**	(+/- IR)*		(+/-)*		(+)*				
CS-42 cell	**0.05%**			(+/-)*		(+)*	(+/-)*			
suspension	**0.25%**			(+/-)*		(+)*				

*: grading at 48-hour inspection
**: grading at 96-hour inspection
Subject prefix "PT" = dermatology outpatient subject
Subject prefix "CO" = non-patient volunteer

tions on this subject's skin. Another point of interest in this subject's patch-test results is that reactions developed to the non-toxic Lake Coolmunda *M. aeruginosa* bloom sample, but no reaction was produced by the toxin-producing *M. aeruginosa* isolate. While the Coolmunda bloom sample was largely a monoculture of *M. aeruginosa*, as with many cyanobacteria blooms there were other cyanobacterial species and genera present in smaller amounts. This leaves open the possibility that this subject has demonstrated hypersensitivity reactions to components other than *M. aeruginosa* in the two bloom samples. Subject PT19 also registered positive responses to both patch series containing *C. raciborskii* and cylindrospermopsin (*C. raciborskii* AWT 205 isolate and North Pine Dam bloom sample). This is interesting in light of the findings by Stewart *et al* [15], which demonstrate that *C. raciborskii* and purified cylindrospermopsin are capable of producing irritant reactions and delayed-contact hypersensitivity in mice.

The principal conclusions from this study are that cutaneous responses to cyanobacteria are uncommon, with only one of 39 subjects demonstrating significant cutaneous responses to cyanobacterial suspensions. Given this patient's diagnosis of atopic dermatitis, and reports in the literature which are suggestive of other features of atopy [2], further research into this matter may benefit from more specific entry criteria to allow investigation of atopic individuals. This sole diagnosis of atopy must be interpreted cautiously, however, in that diagnoses were only available for the twenty outpatients. As the reference group did not have a comprehensive medical history taken, we cannot infer presence or absence of atopic subjects within the reference group.

Weak reactions to *C. vulgaris* were seen on the skin of subject PT19, and possibly one other subject (PT04). *C. vulgaris*, a common and widespread eukaryotic alga, was chosen as a reference material; *Chlorella* spp. are report-

edly allergenic [17,18], although *C. vulgaris* has been promoted as an allergy preventative and has some anti-inflammatory properties [19]. Acute skin symptoms have been reported from exposure to other freshwater and marine eukaryotic microalgae [20,21].

Considering the single subject response to cyanobacterial patch testing, these data were used to determine sample size estimates that would produce with high probability a statistically significant result. Using nQuery Advisor® 4.0 [22], a Fisher's exact test with $\alpha = 0.05$ two-sided significance level will have 80% power to detect the difference between a Group 1 proportion of 0.050 and a Group 2 proportion of 0.001 when the sample size in each group is 167. A study involving over 300 volunteers would be prohibitively large and expensive; a more targeted approach in future to recruit subjects from more at-risk populations awaits further knowledge of the mechanisms of cyanobacterial toxicity by the cutaneous route.

History of skin disease, allergy

As anticipated, the outpatient group contained a higher proportion of subjects with cutaneous disease than the reference group (see Table 3). However, the percentage of subjects reporting hay-fever, asthma and urticarial diagnoses was higher in the reference group, although these differences were not statistically significant. To the extent that future research efforts in this field may need to concentrate on those individuals with atopic illness, recruitment from a dermatology outpatient population may not confer any particular advantage over recruitment from the general population.

Reactions to sodium dodecyl sulfate

Overall, 44% of subjects (n = 17) did not respond to SDS. Some workers have added SDS to their standard allergy patch test series in order to help differentiate between irritant and allergic reactions [23,24]. However, these workers did not appear to have blinded themselves to the location of SDS patches; they were apparently using reactions to SDS as reference irritant responses from which to compare reactions to allergen patches. We suspect that the inclusion of SDS as a positive irritant control may not have been the most appropriate procedure in this diagnostic patch testing study; this matter is discussed further by Stewart [[10] (Chapter 4)].

Rationale for determining cyanobacteria concentrations in patch test wells

Our initial challenge was to determine appropriate doses of cyanobacteria to apply to human skin. Prior to commencing this human volunteer study, preliminary irritant mouse ear swelling work had been done with two cyanobacterial suspensions: *M. aeruginosa* QH/NR/Ma03, 5% w/v and *A. circinalis* non-toxic bloom sample, Gordonbrook Dam, Queensland, 10% w/v (lyophilised cyanobacteria in 75% methanol), with negative results [15]. Rietschel & Fowler [[16] (p. 15)] nominate appropriate steps for testing non-standard contactants: initial test concentrations of 0.1% to 1.0% performed on several volunteers, including the investigator. An autoexperiment was conducted on author IS in May 2001. Eight Finn chambers containing 5% w/v suspensions of *M. aeruginosa* QH/NR/Ma03 and the Gordonbrook Dam bloom sample containing predominantly *A. circinalis* were prepared; each suspension was applied with three vehicles: Milli-Q® water, 50% v/v methanol in Milli-Q® water, and acetone. Lyophilised, powdered *M. aeruginosa* and *A. circinalis* cells were each mixed in petrolatum and placed into two of the Finn chambers. Mild irritant reactions were seen on the aqueous *A. circinalis* suspension site, and on the two petrolatum sites. Because author IS has never suffered from dermatitis, we suspected that the irritant reaction, albeit mild, was probably the result of an artificially high concentration of cyanobacterial cells. So the maximum concentrations of cyanobacteria applied to the skin of volunteers (0.25% w/v) were 20-fold lower than the concentration that elicited a mild irritant reaction on the skin of author IS during pre-testing experiments; 0.25% is also 20 to 40-fold lower than concentrations that failed to elicit observable or measurable reactions on mouse ears during open application experiments for irritancy [15]. We did not proceed with using powdered, lyophilised cyanobacteria mixed in petrolatum because of the anticipated loss of precision in determining doses. It was elected to use aqueous cyanobacterial suspensions for these patch testing studies, as water is the solvent of choice in the vast majority of recreational settings, from which arise reports of acute cyanobacteria-related dermatoses. Concomitant exposure to ethanol can often be observed in Australian recreational environments, but not by the cutaneous route.

General discussion

The findings of this small human study are that cutaneous reactions to cyanobacteria are infrequent, at least in the population we sampled and the dose ranges we used. The work in the accompanying paper by Stewart *et al* [15] complements this study, and demonstrates that purified cylindrospermopsin is capable of eliciting irritant and delayed-contact hypersensitivity reactions in mice. The small number of case and anecdotal reports in the literature also shows that cyanobacteria-associated dermatoses are infrequently reported, although mild, self-limiting illnesses, including pruritic rashes, are likely to be under-reported and under-diagnosed [[2,25] (p. 69)]. However, anecdotal reports of incident-free exposures to high levels of cyanobacteria have also been received [[10] (Chapter 4)]; author IS has tried without success to generate a cutaneous response on his own skin through open application

of concentrated cyanobacterial cells on many occasions, from both field samples and laboratory isolates. Images of field workers demonstrating similarly enthusiastic disregard for occupational health and safety matters can be seen at [26-28].

The commercial sector has not been slow to realise that cutaneous responses to cyanobacteria are not unequivocally hazardous. A Google search using the terms "blue green algae" "soothes" and "skin" reveals a bewildering array of products and services that promise relief from much of what ails you. Many of these products are made from *Arthrospira* sp., a cyanobacterium also known as spirulina. Clinical and research dermatologists will no doubt be pleased to hear about:

Spirulina wrap : Rich in antioxidant vitamins, spirulina is the ultimate nutrient boost. This treatment stimulates and nourishes the skin while promoting a healthy, more vibrant appereance (*sic*). (50 minutes) [29]

So there is still a great deal to learn about cyanobacteria and the skin. To what degree these widespread organisms may affect the health of individuals with atopic and non-atopic allergic disease is unknown, but deserves the attention of researchers. The subject of photoallergy and photoirritancy has not been investigated. Most environmental exposures to aquatic cyanobacteria occur in recreational settings, which correlate strongly with exposure to sunlight, so photic effects should presumably be investigated.

Whether cyanobacteria-associated cutaneous eruptions in susceptible individuals are primarily irritant reactions, immediate hypersensitivity or delayed contact hypersensitivity responses is not at all clear. The picture may turn out to be complex and varied, with similarities to the broad topic of phytodermatitis. Wilkinson and Shaw [30] list the principal presenting features of phytodermatitis thus:

1. irritant contact phytodermatitis – both chemical and physical

2. allergic contact phytodermatitis – both immediate and delayed

3. phytophototoxic dermatitis

4. pseudophytophotodermatitis...

5. allergic contact phytodermatitis with secondary photosensitivity...

Cyanobacteria-related dermatoses may also operate through different molecular mechanisms and may therefore vary in clinical presentation via: individual susceptibility (e.g. atopic phenotype), cyanobacteria profile in waterbodies (different species, genera, cell biomass), cyanotoxins (different types, different mechanisms of toxicity, and variable concentration in waterbodies – i.e. exposure and dose concerns), disruption to barrier function from waterlogged skin, and the influence of ultra-violet irradiation (phototoxic effects or immunosuppressive?).

Conclusion

This pilot study of 39 volunteers identified a single individual with atopic disease who responded to several cyanobacterial preparations applied to the skin by closed patch testing. Dose-response relationships were not observed in this individual, which supports the clinical findings that these were hypersensitivity reactions. This subject developed positive responses to all patch sites containing cylindrospermopsin, whereas none of the remaining 38 subjects showed any response to cylindrospermopsin. This work complements a mouse model study of delayed-contact hypersensitivity that demonstrates cylindrospermopsin is active in mammalian epidermal tissues. Future work into cutaneous effects of cyanobacteria in humans may benefit from improved awareness of cellular and molecular mechanisms to allow more refined targeting of higher-risk populations.

As case reports and epidemiologic studies do not present convincing findings of mass outbreaks of acute cutaneous responses to planktonic freshwater cyanobacteria, the possibility that many such reports are due to hypersensitivity reactions should be considered; these preliminary studies would seem to support this concept.

Abbreviations

CSIRO Commonwealth Scientific and Industrial Research Organisation

i.p. intraperitoneal

LD_{50} lethal dose for 50% of test animals

LPS lipopolysaccharide

ppb parts per billion (μg/L)

ppm parts per million (mg/L)

PVC polyvinyl chloride

SDS sodium dodecyl sulfate (aka sodium lauryl sulfate)

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

IS and IMR initiated the study concept and design. IS grew and processed cyanobacteria, co-ordinated the study, conducted statistical tests and drafted the manuscript. IMR conducted and supervised dermatologist patch test readings. PMW and PJS assisted with study design and statistical advice. GRS supervised the project. All authors read and endorsed the final manuscript.

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