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Technical Memorandum

Evaluation of Cyanobacteria and Cyanobacterial toxins with reference to Selection of Water Quality Criteria for the Karuk Tribe of California.

Prepared for: **Karuk Tribe of California**
Natural Resources Department

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The purpose of this memo is 1) to evaluate current public health criteria for cyanobacteria and their toxins in the Klamath River system of northern California, and 2) using a combination of state agency criteria and site-specific data, recommend cyanobacterial cell density and toxin criteria to protect public health in Karuk tribal areas.

The occurrence of cyanobacterial blooms in the middle Klamath River area is well documented, with the Klamath River from Copco 1 Reservoir (RM 203.1) to Iron Gate Dam (RM 190.1) listed as impaired for toxicity due to the presence of microcystin, a toxin produced by the blue green alga *Microcystis aeruginosa* present in the Project reservoirs (USEPA 2010). In addition, numeric targets for *Microcystis* and associated toxin have been developed by the California North Coast Regional Water Board (NCRWQCB 2010) and were approved by the US Environmental Protection Agency pursuant to Clean Water Act (CWA) Section 303(d)(2).

Although one of the dominant cyanobacterial bloom-formers, *Aphanizomenon flos-aquae*, has not been shown to produce toxins in the Klamath River system, several other species, including *Microcystis aeruginosa* and *Anabaena flos-aquae*[1] have produced toxins at levels harmful to human health. *Microcystis* can produce a potent hepatotoxin known as microcystin that is capable of causing death or severe liver impairment and may also act as a tumor promoter[2]; *Anabaena* can produce anatoxin-a, a potent neurotoxin. Further information and literature reviews on toxicology can be found in OEHHA (2012).

The primary species responsible for the Klamath River toxic blooms, *Microcystis aeruginosa*, consistently produces cell densities and microcystin toxin levels that exceed public health guideline levels both in Copco and Irongate Reservoirs (e.g., Jacoby and Kann 2007, Kann and Corum 2009, Raymond 2010) and downstream of the reservoirs in the Klamath River (e.g., Kann and Bowman 2011, Fetcho 2008). Studies have also shown that bioaccumulation of microcystin has occurred in a variety of fish

[1] In this case *Microcystis* is the primary toxin producer in the Klamath River system; to date anatoxin was detected only in 2005 in Irongate reservoir.
[2] The tumor promoting capability has been demonstrated in laboratory experiments (OEHHA 2012) but the overall role in tumor promotion is less certain.

species and freshwater mussels (Fetcho 2006, 2011; Kann 2008, et al. 2010, et al. 2011; Mekebri et al. 2009, CH2M Hill 2009a, 2009b; Prendergast and Foster 2010).[3]

Current water column public health guideline thresholds being followed by Klamath River management entities for both cyanobacterial cell density and toxin levels are outlined in SWRCB (2010). The SWRCB (2010) decision tree with respect to posting health advisories is shown in Figure 1.

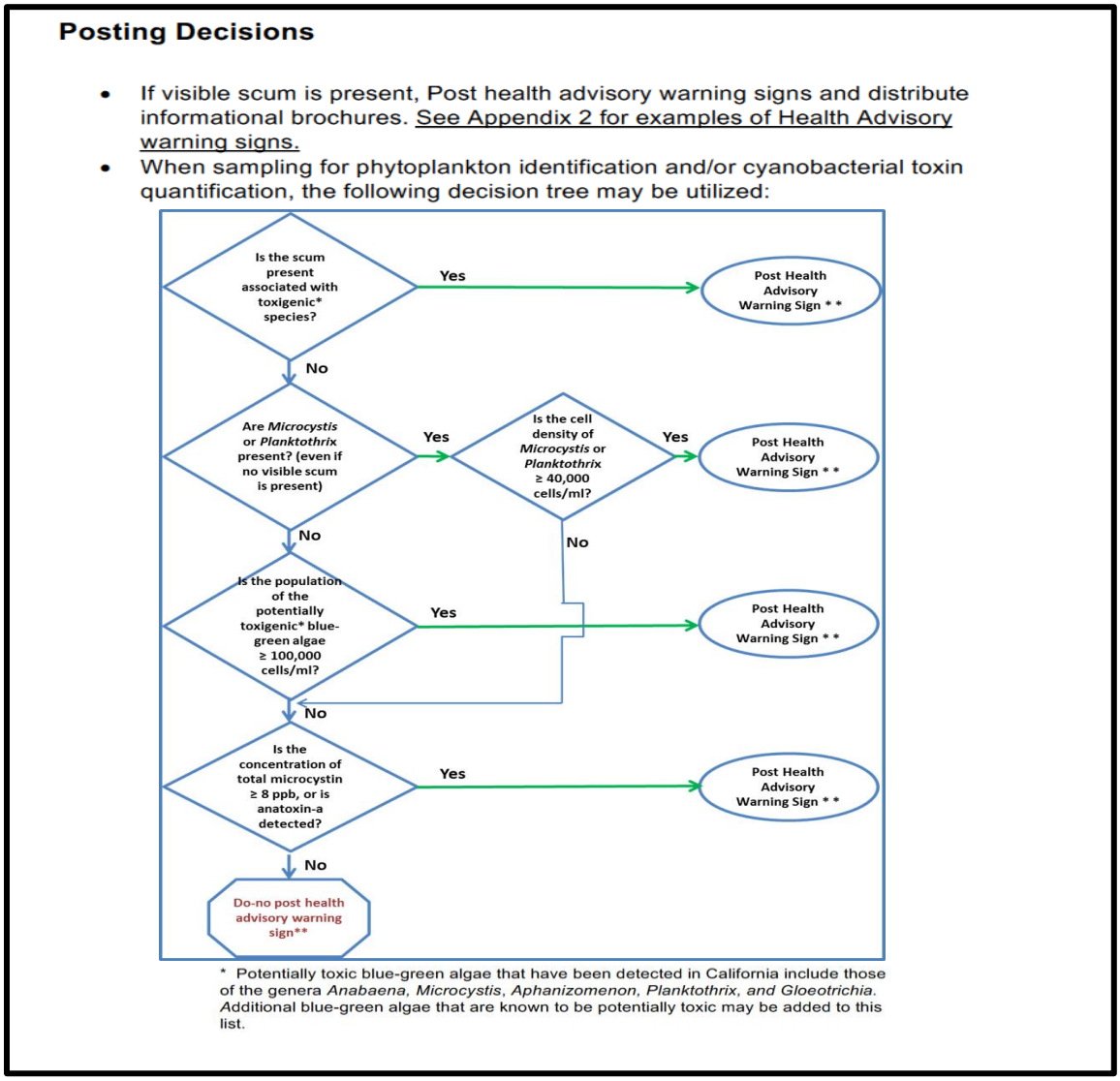

Figure 1. From Blue Green Algae Work Group of the State Water Resources Control Board and Office of Environmental Health and Hazard Assessment: Cyanobacteria in California Recreational Water Bodies Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification DRAFT July 2010.

[3] Microcystin bioaccumulation in Klamath River freshwater mussels is consistent and levels frequently exceed public health guidelines; bioaccumulation in freshwater fish is more variable with levels above public health guidelines occurring in liver and muscle tissue of warm water fish in Copco and Irongate reservoirs in some but not all years. In addition, although microcystin was below detection in muscle and liver tissue in many anadromous fish tested, levels of microcystin have been detected in Irongate hatchery yearling livers as well as in salmon and steelhead livers farther downstream. Although livers are typically not eaten, microcystin levels in some Chinook salmon exceeded public heath guidelines. See cited papers for more detail.

Existing cyanotoxin guidelines, including drinking water and fish/shellfish bioaccumulation guidelines (which are not contained in the SWRCB flow chart) are as shown in Table 1.

Table 1 Public health guidelines for cyanobacterial toxins and cell density currently utilized for the Klamath River and tributaries.

Parameter	Existing Guideline	Rationale for Guideline
Microcystis aeruginosa cell density	<5,000 cells/mL for drinking water <40,000 cells/mL for recreational water	Combination of WHO and SWRCB guidelines--protective of public health
Total microcystin toxin concentration[1]	<1 μg/L total microcystins for drinking water <8 μg/L total microcystins for recreational water	Combination of WHO and SWRCB guidelines--protective of public health
Total potentially toxigenic blue-green algal species[2]	<100,000 cells/mL for recreational water or Cyanobacterial scums	SWRCB guidelines--protective of public health
Anatoxin-a	Positive detection	SWRCB guidelines--protective of public health
Microcystin for Fish/Shellfish Consumption	<26 ng/g (OEHHA 2008a based on Heinze 1999). Previously was <40 ng/g wet weight for Seasonal TDI[3] (which equated to the concentration above which a child should not consume one 8 oz meal per month—OEHHA 2008b).	Combination of WHO, Ibelings and Chorus (2007) and OEHHA (2008a; 2008b)--Protective of public health

[1]While there are numerous congeners of microcystin (e.g., microcystin-LA, RR, and YR) the most extensive toxicological information is available for the microcystin-LR congener. However, the literature indicates that most of these congeners appear to have similar toxicological effects (OEHHA 2012). Therefore, the toxicity criteria apply to the total of all microcystin congeners (if measured separately the concentration of the various congeners is summed), or if ELISA methodology is used then the reported value is already assumed to represent the total.

[2]Includes: *Anabaena, Microcystis*, *Planktothrix, Gloeotrichia* and *Oscillatoria.*

[3]Note that Ibelings and Chorus (2007) also include acute Acute and Lifetime (Chronic) Tolerable Intakes that are 250 ng/g and 4 ng/g, respectively (ng/g = μg/kg).

However, the SWRCB (2010) guideline levels may now be superseded by a recent SWRCB-contracted report by the State of California Office of Environmental Health Hazard Assessment (OEHHA) summarizing algal toxin effects, providing a risk assessment to determine the cyanotoxin concentrations at which no adverse health effects are expected to occur, and suggesting "action levels" (OEHHA 2012)[4].

[4] OEHHA notes that cyanobacteria produce cyanotoxins other than those evaluated in their report and that "cyanotoxins include over 80 similar but distinct microcystins, as well as other toxins". Moreover, they note that the number of identified microcystin analogs has grown significantly and there may be analogs yet to be identified. Thus the OEHHA report does not address all of the important cyanotoxins such as anatoxin-a(s), saxitoxins and other analogs of microcystins. Toxicological criteria are also needed for these cyanotoxins and should be developed in the future.

According to the OEHHA (2012) report the derived action levels are intended to be "***scientifically based health protective "action levels" that may be applied as needed, by local, regional, state or tribal entities throughout California, to reduce (or eliminate) algal toxin exposures".***

OEHHA computed health-based water concentration levels (also known as "action levels"), for people, pets and livestock. Health based concentrations in sport fish and shellfish were also computed. The human water levels are only applicable to incidental exposure through recreational use (applying to water that may be incidentally ingested during recreational activities like water skiing and swimming), they are not intended to apply to treated or untreated water that is intended for drinking, which may be consumed in much larger quantities (OEHHA 2012). The results of the OEHHA (2012) calculations leading to suggested action levels are shown in Table 2.

Table 2. OEHHA health-based water concentration levels (also known as "action levels"), for people, pets and livestock (Office of Environmental Health Hazard Assessment; Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins, 2012).

Action levels for selected scenarios

	Microcystins[1]	Anatoxin-a	Cylindro-spermopsin	Media (units)
Human recreational uses[2]	0.8	90	4	Water (µg/L)
Human fish consumption	10	5000	70	Fish (ng/g) ww[3]
Subchronic water intake, dog[4]	2	100	10	Water (µg/L)
Subchronic crust and mat intake, dog	0.01	0.3	0.04	Crusts and Mats (mg/kg) dw[5]
Acute water intake, dog[6]	100	100	200	Water (µg/L)
Acute crust and mat intake, dog	0.5	0.3	0.5	Crusts and Mats (mg/kg) dw[5]
Subchronic water intake, cattle[7]	0.9	40	5	Water (µg/L)
Subchronic crust and mat intake, cattle[7]	0.1	3	0.4	Crusts and Mats (mg/kg) dw[5]
Acute water intake, cattle[7]	50	40	60	Water (µg/L)
Acute crust and mat intake, cattle[7]	5	3	5	Crusts and Mats (mg/kg) dw[5]

[1] Microcystins LA, LR, RR, and YR all had the same RfD so the action levels are the same.
[2] 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 the acceptability of drinking water concentrations.
[3] Wet weight or fresh weight.
[4] Subchronic refers to exposures over multiple days.
[5] Based on sample dry weight (dw).
[6] Acute refers to exposures in a single day.
[7] Based on small breed dairy cows because their potential exposure to cyanotoxins is greatest. See Section VI for action levels in beef cattle.

The action levels are defined as the "health protective" concentration in the media (for contact recreation or in the case of the Karuk Tribe these would be for ceremonial use as well) or the maximum concentration of cyanotoxins in edible fish and shellfish tissues that a typical consumer (one meal per week) could ingest without exceeding the reference doses (RfD's[5]). Given that the OEHHA (2012) computed RfD represents the maximum dose to which people could be exposed without significant risk of adverse health effects, it appears that the "action levels" in Table 2 would provide the basis for Karuk water quality standards that are most effective for the protection of public health. The wording used for the RfD implies that at concentrations above the 'action levels' that significant risk of adverse health effects could occur. The question then arises as to what 'action' should be taken when these values are exceeded. On p. 10 of the OEHHA (2012) document it sates "Public health officials need a basis to prevent or warn of exposures to toxic chemicals that may lead to adverse health effects". Thus at a minimum, it appears that exposure warnings (possibly similar to the public health advisories currently issued) would be issued by appropriate agencies when the action levels are exceeded.

Clearly if these OEHHA action levels are adopted as guidelines for posting public health advisories then both microcystin levels and associated cell densities will be substantially lower than the currently utilized SWRCB (2010) guidelines (for example the OEHHA microcystin action level value of 0.8 µg/L is 10x lower than the SWRCB value of 8 µg/L). However, the bases for these values as well as interpretation of the "action levels" should be made in the context of the different toxicological studies and toxic end points utilized by OEHHA compared to the World Health Organization (WHO), SWRCB (2010), and to Oregon and Washington. Oregon is specifically included here because earlier Oregon guidelines (OHA 2005) for microcystin were adopted by California (SWRCB 2010). For a review and comparison of the OEHHA (2012) action levels with criteria from WHO, SWRCB, Oregon, and Washington see Appendix I, below.

Although the previous SWRCB guidelines were based on older Oregon criteria[6] for posting water bodies, both new Oregon criteria (Oregon 2012) and OEHHA (2012) "action levels" are based on a toxicological study (Heinze 1999) that was not available when the earlier Oregon WHO-based criteria were developed. However, although both Oregon and OEHHA utilize Heinze (1999) as their basis, a different toxic end point is used by each entity. Specifically, the 2012 Oregon value is related to an endpoint concentration having an impact (liver lesions) on the test organisms, while the OEHHA (2012) value was developed from an endpoint value where there was essentially no impact to the test organisms (see Appendix I for a more detailed description). Uncertainty factors of 1000 were then applied to both of these endpoints to derive final criteria[7]. So where Oregon's action is to post the water when criteria are exceeded; California needs to consider what <u>action</u> to implement when waters reach the maximum level where people could be exposed without significant risk of adverse health effects (as above the RfD computed by CA "represents the maximum dose to which people could be exposed without significant risk of adverse health effects").

Although recreational criteria in much of the rest of the United States and other countries tend to be 4 µg/L or higher for total microcystin (Chorus 2012), these are based on older WHO criteria and are not based on the Heinze (1999) study that Oregon and OEHHA[8] used. Thus, since both Oregon Health Authority and California OEHHA consider Heinze to be a more suitable toxicological study than those

[5] According to OEHHA (2012) p11. "The RfD represents the maximum dose to which people could be exposed without significant risk of adverse health effects."
[6] Which were based on WHO Tolerable Daily Intake Values
[7] In this fashion Oregon derived a microcystin concentration that was 1,000 times lower than the concentration shown to cause microscopic liver lesions in 6 of 10 rats. OEHHA derived a microcystin concentration that was 1,000 times lower than the concentration that is estimated to have essentially no effects on the rats.
[8] The US Environmental Protection Agency (USEPA) in a draft report also used Heinze (1999) and derived a similar value to OEHHA (USEPA 2006).

previously utilized by the WHO (see OEHHA and Appendix I below), the question remains as to which toxicological endpoint to base public health advisories upon?

As described above and in Appendix I, the current Oregon public health guideline value of 10.0 µg/L is based on a different toxic endpoint than used by OEHHA, and reflects a level that included effects on the test organisms (a LOAEL of 50 µg/kg/day). However, even if one uses the Oregon endpoint, at a minimum the California water exposure assumptions would be applied. Thus, when revised to reflect water exposure assumptions recommended by OEHHA[9] (see Appendix I, below), a public guideline value of 6.0 µg/L is arrived at. Although the later value of 6.0 µg/L includes the Oregon endpoint based on a level that included effects on the test organisms (a LOAEL of 50 µg/kg/day), the final TDI used by Oregon (0.05 µg/kg/day) includes a combined safety factor of 1000x. However, since the OEHHA action level of 0.8 µg/L appears to represent the "maximum dose to which people could be exposed without significant risk of adverse health effects", then a value of 6.0 µg/L may not be protective of public health.

Previous WHO-based numeric targets of 20,000 cells/ml for *Microcystis aeruginosa* and 4 µg/L for microcystin were set by the North Coast Regional Board (NCRWQCB 2010). These TMDL target values were set to protect against beneficial use impacts and were therefore set at the level of the WHO low probability of health effects levels. As noted by NCRWQCB (2010), values above in Table 1 (40,000 cells/ml *Microcystis* and 8 µg/l microcystin) are used to take action (public health posting or listing) when impairment is occurring and represent a moderate level of health effects. Water quality standards are intended to protect against beneficial use impacts and thus may be lower than values used to take action. For example, by the time a water body is posted as being unsafe for water contact, impairment has already occurred. Since the newer Heinze-based criteria and action levels are not presented in terms of "low" or "moderate" probabilities of adverse health effects, the OEHHA 0.8 µg/L action level may again be supported as an appropriate water quality standard to prevent impairment.

Additionally, an important point to consider is that even at very low to non-detectable ambient concentrations of microcystin in the Klamath River, bioaccumulation of microcystin in freshwater mussels has exceeded various public health guideline levels (Kann et al. 2009). Thus, bioaccumulation dynamics should be considered when determining water quality standards, and such dynamics provide support for lower ambient values, because water contact standards would not necessarily result in meeting tissue standards for freshwater mussels.

Another factor to consider when setting water quality standards for microcystin is the inherent temporal and spatial variability in the distribution of microcystin toxin or *Microcystis* colonies. For example, four samples collected in succession at Orleans (Sep 2nd 2012 during the pulse flow event) showed fine scale spatial and temporal variability in microcystin concentration (values were 6.6, 8.6, 7.5, and 3.8 µg/l). In this scenario, the water would have been considered safe if the initial value of 6.6 µg/l had been the only value collected, even though the second sample exceeded the 8 µg/l public health guideline value. Thus, when cell densities are elevated and microcystin concentrations approach but may not exceed public health thresholds, caution should still be exercised with respect to water contact[10].

Diel studies performed by the Karuk Tribe in 2013 also illustrate this point, with error bars indicating that samples collected at the same point time are variable with respect to the public health threshold, and that mean values can vary substantially over a 24 hour period (Figure 2). The 1000-fold safety factor used in setting the guideline may provide some latitude in these instances; however, ensuring that water contacted by the public is truly below a guideline or standard would require a lower and more conservative value.

[9] OEHHA provides extensive documentation for the exposure assumptions utilized to develop "action levels"

[10] In this particular instance and in the diel study, the river had been previously posted, but this may not always be the case.

This would be particularly important if the Heinze (1999) LOAEL is used as the toxic endpoint, whereas using the OEHHA RfD as the toxic endpoint provides a much higher degree of latitude with respect to spatial/temporal variability of toxin in the river environment. Setting the standard lower than the value intended to protect public health is similar to the rationale used by the NCRWCB (2010) in choosing a level intended to prevent impairment.

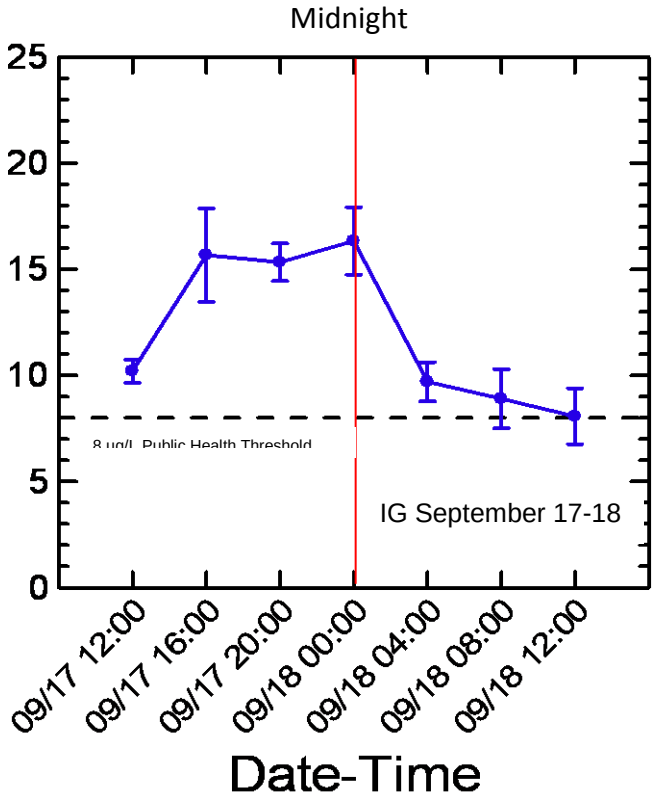

Figure 2. Karuk Tribe Diel Microcystis/microcystin study performed below Irongate dam, September 17-18, 2013. Circles and error bars represent the mean and standard error of 3 replicate sampled taken on the hour using and ISCO automated sampler.

Based on these factors and the above discussion, the Karuk Tribe's proposed public health protection protocol is as follows 1) issue general media outreach and public advisory[11], and begin regular public health monitoring (if it has not already commenced) when total microcystin levels exceed the 0.8 µg/L "action level", and 2) issue an additional water contact warning or alert (with specific media outreach and posting of water access areas with signs stating that water contact should be avoided) when total microcystin levels exceed 5x the "action level" or 4.0 µg/L[12]. To avoid complacency, an additional media outreach would occur when levels reach 10x the "action level" or 8 µg/L (this would be stated in media outreach).

[11] Such outreach could include language such as: Klamath River levels of the cyanobacterial toxin microcystin have exceeded public health "action levels" **(representing the maximum dose to which people could be exposed without significant risk of adverse health effects)** and caution should be taken to minimize ingestion when in contact with water ***and when eating freshwater mussels harvested from the river.***

[12] Specific language in the media would state that levels are now 5x the "Action Level" or maximum dose to which people could be exposed without significant risk of adverse health effects. The 4.0 µg/L level also has the advantage of being consistent with current TMDL targets for the Klamath River.

Relationship between microcystin toxin and *Microcystis* cell density

Specific algal toxin measurements and comparison to subsequent public health thresholds provide the most accurate means of determining public health risk. However, the presence of cyanobacteria, whether visually as scums, or microscopically identified, can also be utilized as public health criteria (SWRCB 2010). As noted by OEHHA (2012), cyanobacterial counts may not provide adequate information since it is the toxins and not the cyanobacteria that cause severe toxicity.

For example, cyanobacterial counts can overestimate the risk of cyanotoxin poisoning if cyanobacteria are present but not producing toxin and they can also underestimate the risk of cyanotoxin poisoning because cyanotoxins may persist in the water after a cyanobacterial bloom has subsided and is no longer visible (OEHHA 2012). Moreover, previous site-specific data analyses have shown variable ratios of toxin produced per unit cyanobacteria (e.g., Kann and Corum 2008; Kann and Bowman 2011), and genetic studies show that the presence of toxin producing genotypes can vary seasonally in the Klamath River (Bozarth et al. 2010).

Thus if toxin measurements are readily available then those would provide the preferable means to evaluate public health thresholds; however in the event that such measurements are not readily available, cyanobacterial cell densities[13] (counts) can also suffice for comparison to standards. Moreover, because some species of cyanobacteria can produce multiple toxins and often not all potential toxins are measured, microscopic identifications and measured cell density may also be used to evaluate public safety. Furthermore, ecological monitoring for phytoplankton is often routinely performed, and in the absence of toxin data can be used to inform public health advisories.

Once a specific toxin threshold has been determined, either a generalized relationship between cell density and toxin can be utilized[14], or preferably (if sufficient data exist) site-specific information depicting the relationship between cell density and measured toxin can be utilized to determine protective cyanobacterial cell densities (e.g., those that minimize the probability of exceeding the determined public health thresholds for toxin).

In the case of the Klamath River, adequate site-specific data do exist[15] and relationships between cell density and toxin were previously developed (e.g., Kann and Corum 2009; Kann and Bowman 2011); such relationships were then utilized by the NCWQCB in their TMDL recommendations (NCWQCB 2010). For the purposes of this technical memorandum those relationships were subsequently updated utilizing the full 2005-2012 dataset.

Because the most common toxin producing species in the Klamath River system is *Microcystis aeruginosa*, the relationship between *Microcystis aeruginosa* and microcystin toxin is the one focused on herein[16]. The generalized relationship between *Microcystis* cell density and microcystin for all years is shown in Figure 3 with limit lines shown for the SWRCB 40,000 cells/mL *Microcystis* and 8 µg/L microcystin public health thresholds, as well as the WHO low probability of adverse health effect levels of 20,000 cells/mL *Microcystis* and 4 µg/L microcystin. Similar plots are shown for individual years (Figure 4). These plots indicate that although there is variability overall and among years (for example the relationship in 2010 showed reduced variability compared to 2007 or 2008), in general there is a positive and statistically significant relationship between cell count and toxin in the Klamath River system.

[13] Which are often routinely collected as part of water quality monitoring programs
[14] Such as those utilized by the World Health Organization (WHO) and SWRCB.
[15] But only for *Microcystis aeruginosa* and total microcystin
[16] Note that this is total microcystin as measured by ELISA as opposed to any individual variant or congener.

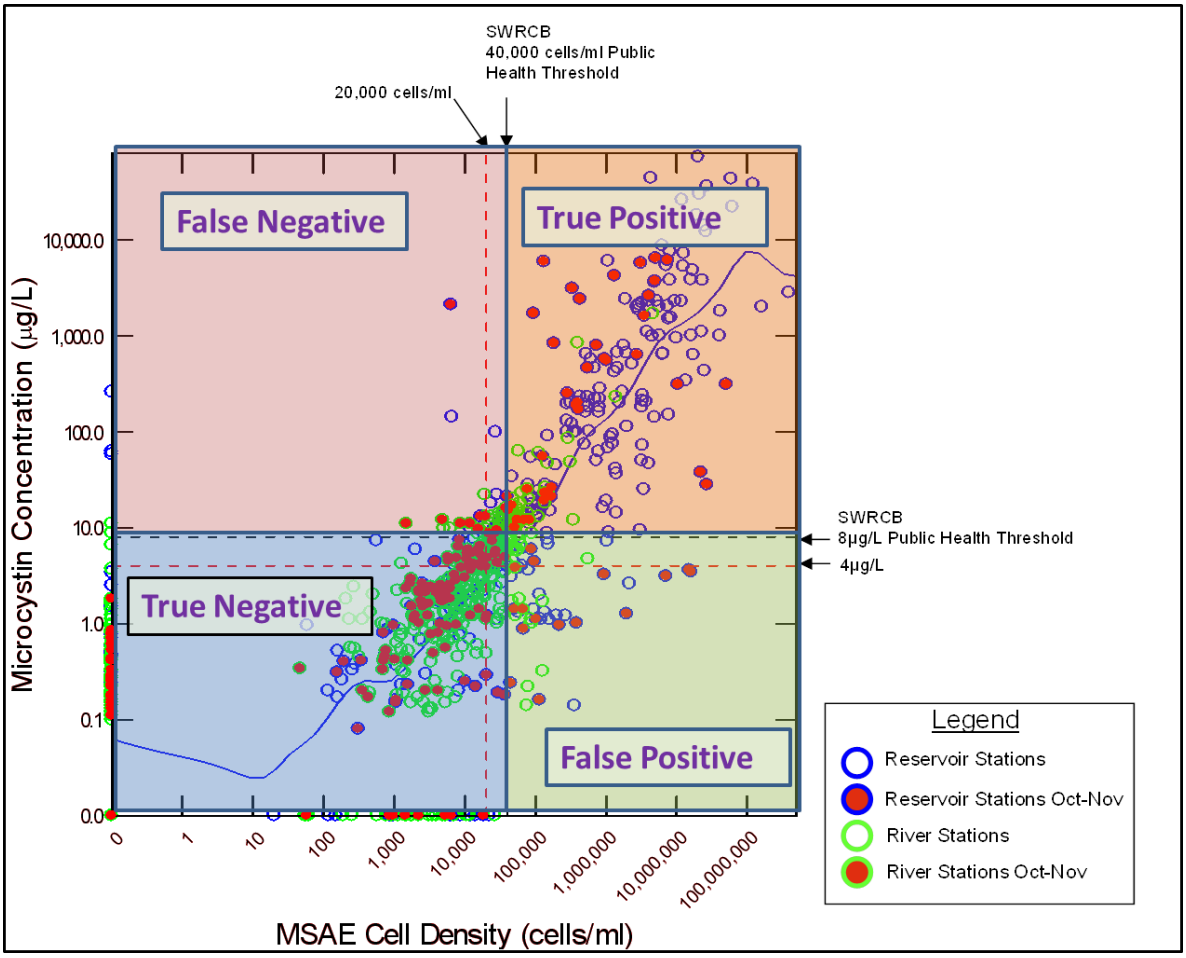

Figure 3. Relationship between MSAE cell density and microcystin toxin concentration for standard reservoir and river stations 2005-2012; shown with distance weighted least squares (DWLS) smoother. Data source: Karuk Tribe and PacifiCorp (www.pacificorp.com/es/hydro/hl/kr.html). Data in this figure represent all available data to determine the cell density/toxin relationship. In some false negative instances the river may have been posted based on previous sampling data.

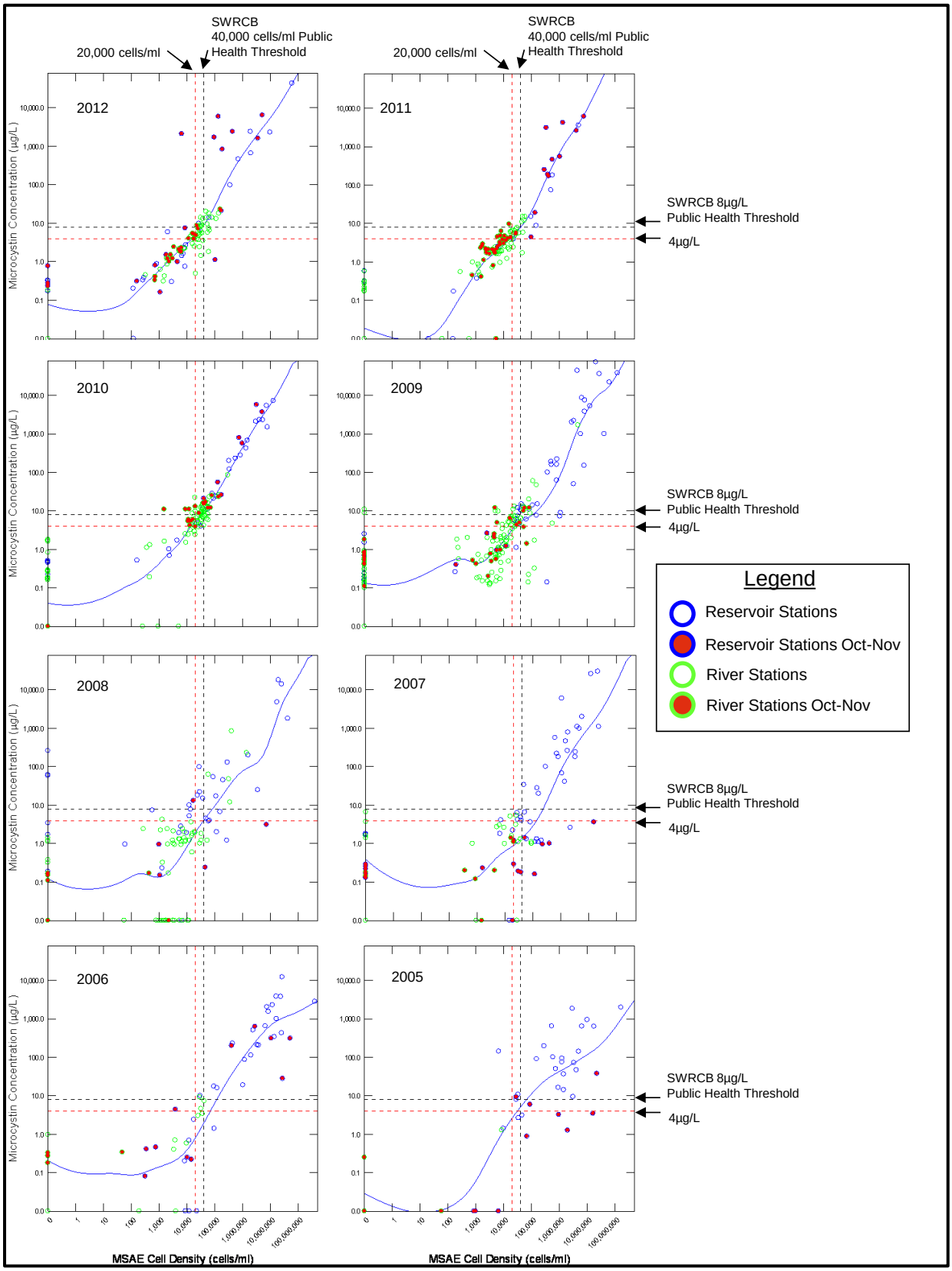

Figure 4. Relationship between MSAE cell density and microcystin toxin concentration for standard reservoir and river stations 2005-2012; shown with distance weighted least squares (DWLS) smoother. Data source: Karuk Tribe and PacifiCorp (www.pacificorp.com/es/hydro/hl/kr.html).

As noted by NCWQCB (2010), values in the upper left hand quadrant are false negative measurements, representing the potential risk to public health with adoption of a given numeric cell density/toxin threshold[17]. For example, in Figure 3 and Figure 4 the upper left quadrant delineated by the red dashed lines (20,000 cells/mL *Microcystis* and 4 µg/L microcystin relationship) or the black dashed lines shows observations that have concentrations of microcystin exceeding the threshold criteria of either 4 or 8 µg/L, even when *Microcystis aeruginosa* cell density is less than either 20,000 or 40,000 cells/mL. In other words because the expectation is that when cell density is less than either 20,000 or 40,000 cells/mL, toxin will be less than either 4 µg/L or 8 µg/L, when the respective toxin values are higher than expected such values are termed false negatives[18]. The ultimate goal in choosing cell density thresholds is then avoidance or minimization of obtaining a false negative result because the implication is that water contact or exposure is safe, when in fact it is not.

Regression analysis also shows that in many years and for the overall relationship that the mean microcystin predicted at 40,000 cells/mL is greater than 8 ppb (Figure 5 and Table 3). Particularly in the later years (2010 through 2012), the mean predicted values were ~5 µg/L higher than expected. However, the regression predicts the mean value, and as shown by the prediction bands, variability around the mean is high (Figure 5). For example, although the predicted overall mean microcystin is 8.3 µg/L at 40,000 cells/ml, the prediction bands indicate a predicted microcystin value ranging between 0.5 µg/L and 150 µg/L (Figure 5).

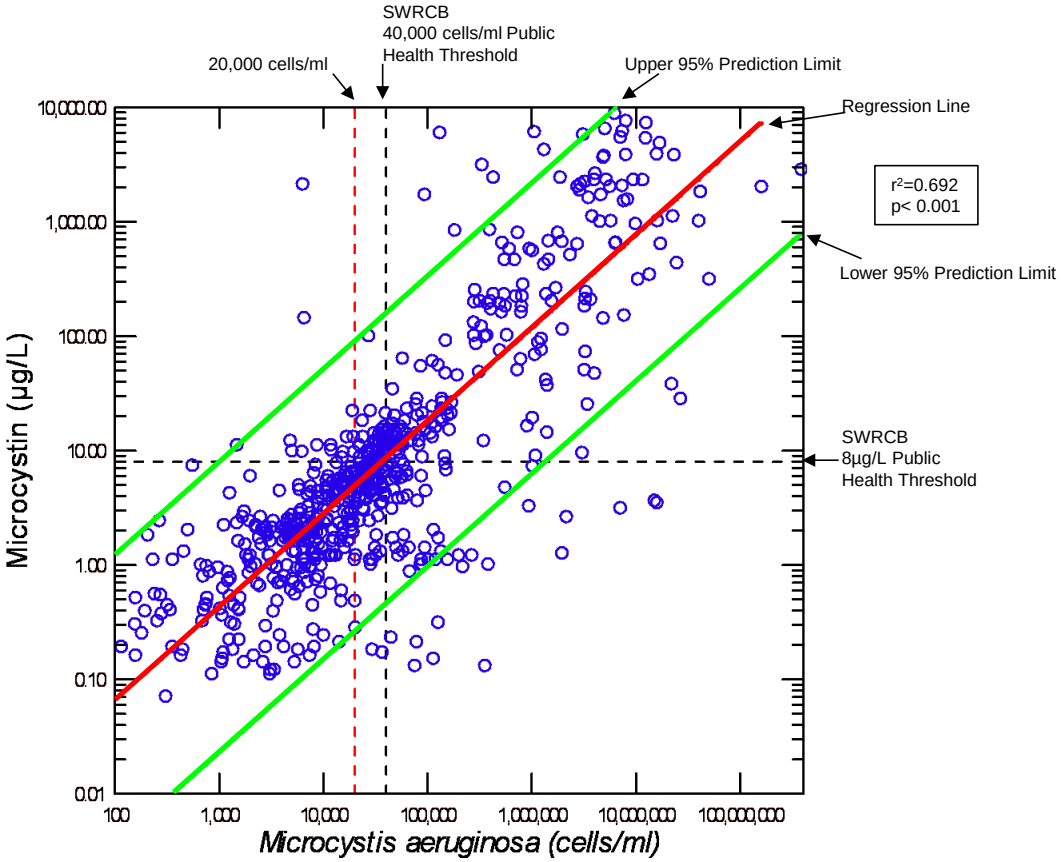

Figure 5. Regression analysis between MSAE cell density and microcystin toxin; May-November, 2005-2012. Includes only observations when MSAE was detected.

[17] Although in some cases the reservoirs or river may be posted based on previous data.

[18] A false negative is a result that appears negative when it is not; or in this case microcystin toxin concentration is deemed safe when in fact it is not.

Table 3. Regression statistics for log-log relationships between MSAE and microcystin in the Middle Klamath River System. Includes only observations when MSAE was detected.

Year	N	R^2	constant	slope	p value	Predicted Microcystin at 40,000 cells/mL Microcystis
All Years	655	0.692	-2.82	0.812	<0.001	8.3
All Years (August only)	188	0.895	-3.399	0.924	<0.001	7.1
2012	94	0.717	-3.173	0.937	<0.001	13.8
2011	101	0.845	-3.215	0.946	<0.001	13.8
2010	101	0.876	-3.219	0.946	<0.001	13.6
2009	145	0.73	-3.39	0.92	<0.001	7.0
2008	71	0.601	-2.391	0.709	<0.001	7.4
2007	65	0.599	-3.747	0.927	<0.001	3.3
2006	45	0.83	-2.932	0.805	<0.001	5.9
2005	33	0.278	-1.278	0.481	<0.001	8.6

Clearly the high variability associated with these regression-based predictions can obscure more specific thresholds; however, the high level of correlation between cell count and microcystin concentration provides the basis for utilizing other non-parametric probability methods (e.g., Kann and Smith 1999) to compute the percent exceedances of a particular level of microcystin concentration at a given cell density.

For example, using this method and evaluating for the SWRCB (2010) guideline of 8 µg/L, the analyses indicate that for the Klamath River Stations there was a 48% probability of exceeding 8 µg/L at a *Microcystis* cell density of 40,000 cells/ml (**Figure 6**). Similarly there was a 10% probability of exceeding 8 µg/L at a *Microcystis* cell density of 20,000 cells/ml for the river-only stations, and a 32% probability for the reservoir-only stations. These plots show that the risk of exceeding 8 µg/L begins to increase sharply at ~10,000 cells/ml and that the 40,000 cells/ml guideline is clearly not protective of public health (using the current SWRCB guideline or the 10x the OEHHA "action level'of 8 µg/L) if the intent is to post or provide advisories in the absence of toxin information.

Similar analyses computed for 6 µg/L microcystin (see derivation of this number above), 4 µg/L (the Karuk Tribe's water contact posting level and the WHO value for low probability of adverse health effects), and 0.8 µg/L (the OEHHA action level) are shown in Figures 6 through 8. Comparatively, the analyses indicate that for the Klamath River Stations probabilities were 58%, 36%, and 10% for exceeding 6 µg/L at *Microcystis* cell densities of 40,000, 20,000, and 10,000 cells/ml (Figure 7); 84%, 56%, and 20% for exceeding 4 µg/L at *Microcystis* cell densities of 40,000, 20,000, and 10,000 cells/ml (Figure 8); and 99%, 96%, and 84% for exceeding 0.8 µg/L at *Microcystis* cell densities of 40,000, 20,000, and 10,000 cells/ml (Figure 9). Note that the positive probabilities at zero *Microcystis* (particularly obvious in the 0.8 µg/L plot), primarily reflect instances when blooms have died back but soluble microcystin is still present in the water column. *Microcystis* cell density would have to be lower than 1000 cells/ml for substantial reduction of the probability of exceeding the 0.8 µg/L threshold.

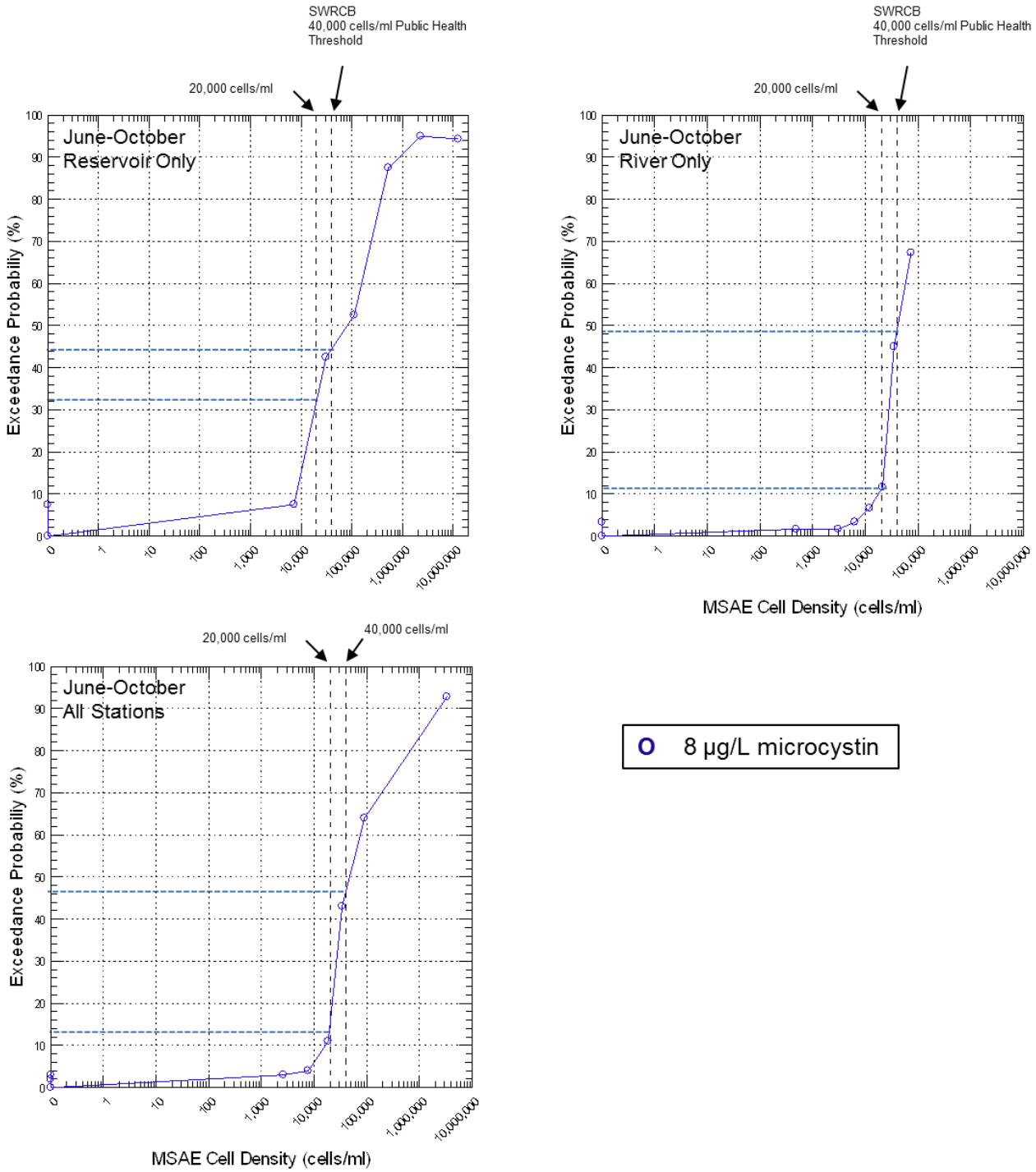

Figure 6. Probability of exceeding the WHO/SWRCB public health microcystin toxin level of 8µg/L at varying MSAE cell density; reservoir-only Jun-Oct (a), river only Jun-Oct (b), and all stations Jun-Oct (c) in Copco and Iron Gate Reservoirs and the Klamath River, 2005-2012. Exceedance probabilities are computed using nonparametric cross-tabulation methods described in Kann and Smith (1999).

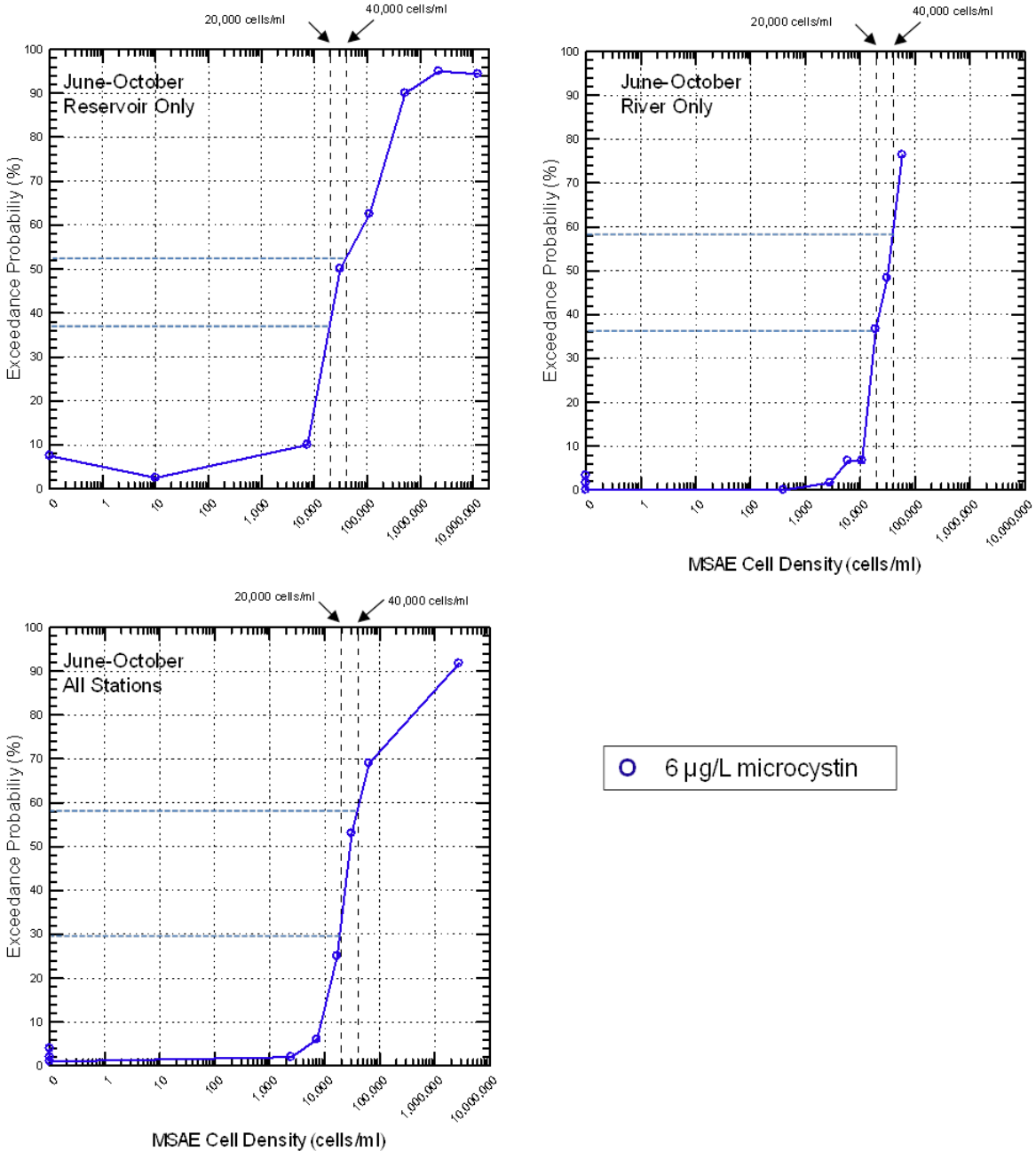

Figure 7. Probability of exceeding the WHO public health microcystin toxin level of 6µg/L) at varying MSAE cell density; reservoir-only Jun-Oct (a), river only Jun-Oct (b), and all stations Jun-Oct (c) in Copco and Iron Gate Reservoirs and the Klamath River, 2005-2012. Exceedance probabilities are computed using nonparametric cross-tabulation methods described in Kann and Smith (1999).

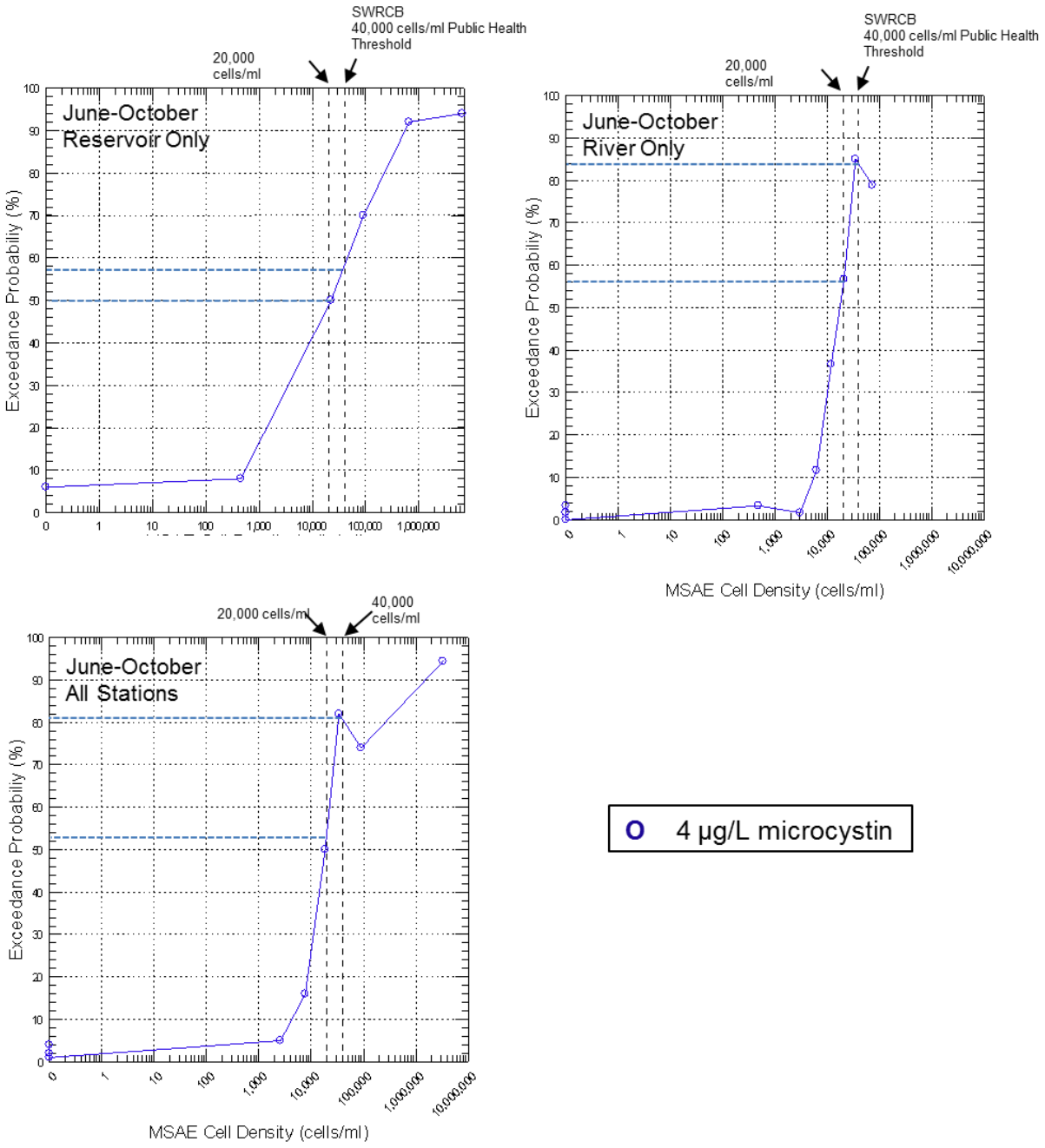

Figure 8. Probability of exceeding the WHO public health microcystin toxin level of 4µg/L at varying MSAE cell density; reservoir-only Jun-Oct (a), river only Jun-Oct (b), and all stations Jun-Oct (c) in Copco and Iron Gate Reservoirs and the Klamath River, 2005-2012. Exceedance probabilities are computed using nonparametric cross-tabulation methods described in Kann and Smith (1999).

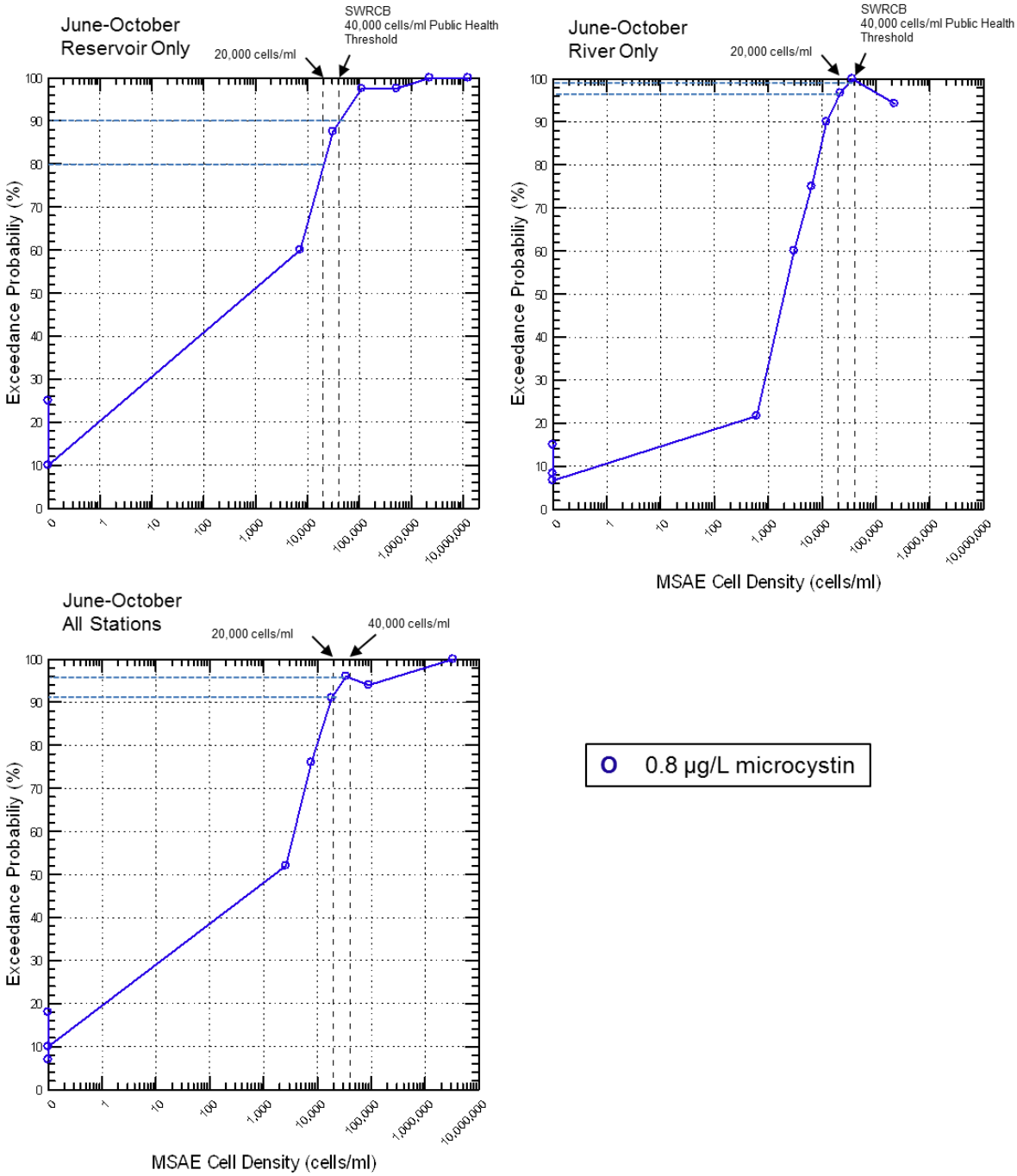

Figure 9. Probability of exceeding the WHO public health microcystin toxin level of 0.8µg/L at varying MSAE cell density; reservoir-only Jun-Oct (a), river only Jun-Oct (b), and all stations Jun-Oct (c) in Copco and Iron Gate Reservoirs and the Klamath River, 2005-2012. Exceedance probabilities are computed using nonparametric cross-tabulation methods described in Kann and Smith (1999).

Because the above plots require an algorithm that limits evaluation of the exceedance probability at a specific threshold[19], a method that includes rolling intervals (i.e., intervals are not independent but include all successive intervals – see Kann and Smith 1999 for specific methodology) allows for specific targets to be evaluated as well as a clear determination of infection points in the various relationships.

For, example, this method shows that the independent intervals (red "plus" symbols) provide a good description of the overall trend depicted by the rolling intervals (open circles), and shows the *Microcystis* level beyond which the various microcystin exceedance probabilities increase rapidly (Figure 10). The probability of exceeding both 6 µg/L and 8 µg/L microcystin increases rapidly above 10,000 cells/ml, and as noted above, the probability of exceeding 6 µg/L increases to 36% by the time cell density reaches 20,000 cells/ml. Thus, the inflection point occurring at 10,000 cells/ml of *Microcystis* provides a protective level beyond which the probability of exceeding the 6 µg/L or 8 µg/L critical microcystin levels rapidly increases. Similarly, for the new OEHHA-based Karuk microcystin level of 4 µg/L[20], the inflection point occurs at ~5000 cells/ml.

Depending on which toxin threshold is evaluated (8 µg/L, 6 µg/L, 4 µg/L, or 0.8 µg/L) the cell density varies accordingly, but it is clear that even for the currently utilized 8 µg/L level, cell densities lower than those recommended by the WHO and SWRCB[21] are necessary if they are intended to be protective of public health by reducing the risk of exceeding critical toxin levels. The variability in these relationships underscores the preference for using actual toxin measurements rather than cell density. However, such cell density-toxin relationships still have great utility for evaluating exposure risks, and as noted by Paerl and Otten (2013) such relationships are needed to characterize the exposure risks for all microcystins and the numerous other cyanotoxins endemic in many waterbodies worldwide.

Given these cell density/toxin relationships and the discussion above on the OEHHA (2012) "action levels", Table 4 provides the proposed water quality guidelines for algal toxins and potentially toxigenic cyanobacteria. If de-posting is based on toxin only, then levels would need to be below 4 µg/L for two successive weeks. If de-posting is based on cell density then densities need to be below 5,000 cells/ml for two successive weeks.

[19] Exceedance probabilities are computed for independent, non-overlapping intervals and are plotted on the median *Microcystis* values for each interval. Although plotting on the interval median allows the general shape and behavior of the relationship to be determined, it does not allow for a specific numeric threshold to be as easily determined.

[20] Based on a level of 5x the OEHHA "action level" as described above

[21] As noted above, these levels are 20,000 cells/ml (for the 4 µg/L low probability of adverse health effect level) and 40,000 cells/ml (for the 8 µg/L moderate probability of adverse health effect level)

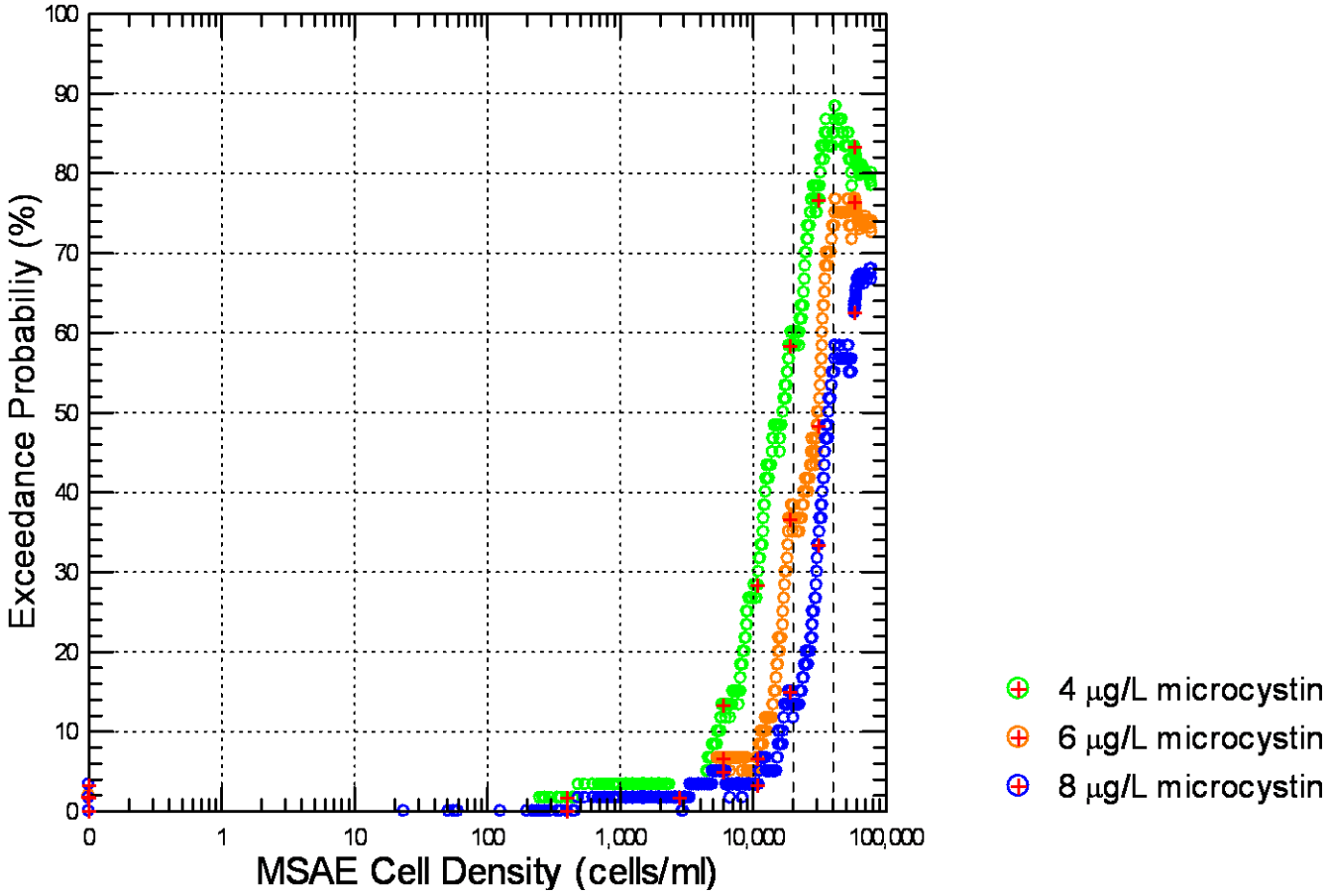

Figure 10. Probability of exceeding three microcystin levels (0.4 µg/L, 6 µg/L, and 8 µg/L) at varying MSAE cell density; Klamath River only, Jun-Oct, 2005-2012. Circles represent rolling non-independent intervals; "plus" symbols show the original independent intervals from Figures 5-8, above. Exceedance probabilities are computed using nonparametric cross-tabulation methods described in Kann and Smith (1999).

Table 4. Proposed Karuk Tribe public health guidelines for cyanobacterial toxins and cell density for the Klamath River and tributaries.

Parameter	Proposed Guideline (in bold type)	Rationale for Guideline
Microcystis aeruginosa cell density	**Below detection for drinking water**	The Minnesota (2012a, 2012b) Heinze-based BMDL short-term non-cancer "Health Based Value" of 0.04 μg/L essentially does not allow for the detection of any cells.
	1,000 cells/mL for recreational water-- Initial media outreach and general informational signage. Begin routine monitoring.	Cell density corresponding to OEHHA "Action Level"
	5,000 cells/mL for recreational water Additional media outreach and specific public health postings that warning against water contact due to levels that are 5x the OEHHA "action level"	Cell density corresponding to 5x OEHHA "Action Level"
	10,000 cells/mL for recreational water Repeat Media outreach and specific public health postings warning against water contact due to levels that are 10x the OEHHA "action level"	Cell density corresponding to 10x OEHHA "Action Level"
Total microcystin toxin concentration[1]	**0.04 μg/L total microcystins for drinking water[2]**	Minnesota (2012a, 2012b) Heinze-based BMDL short-term non-cancer "Health Based Value" of 0.04 μg/L.
	0.8 μg/L total microcystin for recreational water- Initial media outreach and general informational signage. Begin routine monitoring.	OEHHA "Action Level"
	4.0 μg/L total microcystin for recreational water Additional media outreach and specific public health postings that warn against water contact due to levels that are 5x the OEHHA "action level"	5x OEHHA "Action Level"
	8.0 μg/L total microcystin for recreational water Repeat media outreach and specific public health postings warning against water contact due to levels that are 10x the OEHHA "action level"	10x OEHHA "Action Level"
Total potentially toxigenic blue-green algal species[3]	**100,000 cells/mL for recreational water or cyanobacterial scums**	WHO/SWRCB guidelines

Anatoxin-a	**90 µg/L**	OEHHA (2012)
Cyanotoxins for Fish/Shellfish Consumption	**10 ng/g microcystins; 5000 ng/g anatoxin; 4 ng/g cylindrospermopsin (OEHHA 2012) (wet weight)**	OEHHA (2012)

[1]While there are numerous congeners of microcystin (e.g., microcystin-LA, RR, and YR) the most extensive toxicological information is available for the microcystin-LR congener. However, the literature indicates that most of these congeners appear to have similar toxicological effects (OEHHA 2012). Therefore, the toxicity criteria apply to the total of all microcystin congeners (if measured separately the concentration of the various congeners is summed), or if ELISA methodology is used then the reported value is already assumed to represent the total.
[2]Note that this value is also based on the older WHO studies, and although OEHHA (2012) did not evaluate drinking water "action levels", the Minnesota Department of Health (2012a, 2012b) utilized the same Heinze-based BMDL of 0.0064 mg/kg/day that OEHHA used to arrive at a short-term non-cancer "Health Based Value" of 0.04 µg/L.
[3]Includes: *Anabaena, Microcystis*, *Planktothrix, Gloeotrichia* and *Oscillatoria*

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Appendix I: Review and Comparison of Regional Public Health Guideline Values for Microcystin.

The following supplement to the technical memorandum provides a comparison of California, Oregon, and Washington documents relating to public health guideline values for the hepatotoxin microcystin. The documents include:

1) the recent California Office of Environmental Health Hazard Assessment (OEHHA): **Toxicological Summary and Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins (OEHHA 2012**);
2) the California State Water Resources Control Board (SWRCB) and OEHHA: **CA's Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification, July 2010 Draft (SWRCB 2010)**;
3) the Oregon Health Authority: Public Health Advisory Guidance for Toxigenic Cyanobacteria in Recreational Waters (OHA 2005);
4) the more current Oregon Health Authority: **Oregon Harmful Algae Bloom Surveillance (HABS) Program Public Health Advisory Guidelines: Harmful Algae Blooms in Freshwater Bodies (OHA 2012)**; and
5) the Washington State Division of Environmental Health Office of Environmental Health Assessments: **Washington State Recreational Guidance for Microcystins (Provisional) and Anatoxin-a (Interim/Provisional) (WSDH 2008)**.

The impetus for the following comparison stems from among-state differences in public health guideline levels for microcystin, including new OEHHA (2012) "action levels" which are 10X lower than currently utilized SWRCB (2010) guideline levels for public health posting. The earlier state guidelines were generally adapted from World Health Organization (WHO) guidelines as described in Chorus and Bartram (1999). Original thresholds from WHO were based on mouse studies described in Fawell et al. (1994) and Fawell et al. (1999). State guideline comparison highlights are as follows:

1) The new OEHHA (2012) document utilizes a study by Heinze (1999) (as opposed to the Fawell et al. studies utilized by WHO) for their microcystin risk assessment, and instead of using the lowest observable adverse effect level (LOAEL) of 50 µg/kg/day (as Oregon does—see below); they estimate, using an EPA Benchmark Dose (BMD) approach, the dose associated with the 95% lower confidence limit on a 10% response rate to arrive at 6.4 µg/kg/day. This estimates the lowest microcystin concentration that might result in a 10% response rate (the point at which up to 10% of test animals are expected to be affected). The concentration of 6.4 µg/kg/day is termed the BMDL, and is also referred to as the Point of Departure. The rationale for computing the BMDL is that at the LOAEL of 50 µg/kg/day, 6 of 10 rats already had microscopic liver lesions, so presumably using that as the starting point would not be as protective of public health.

   OEHHA also notes that their BMD approach has limitations because only two dose levels were used in the study, and the BMDL is well outside of the dose range tested. However, they also note that an alternative standard protocol of dividing the LOAEL, 50 µg/kg-d in Heinze (1999), by 10 to estimate a NOAEL of 5 µg/kg-d provides a very similar point of departure as achieved using the BMD approach (6.4 µg/kg-d). Excerpt from p. 15 of the OEHHA document:

"The incidence of microscopic liver lesions was input into the EPA benchmark dose (BMD) software (version 1.3.2). This software fits various mathematical models to the dose-response data to estimate the dose associated with a 10% response rate (the BMD) and a 95% lower confidence limit on the BMD (BMDL). The log-probit fit of the data was determined to be the best fitting model and this resulted in a BMDL estimate of 6.4 µg/kg-d. OEHHA's use of the BMD approach here does have limitations: only two dose levels were used in the study and the BMDL is well outside of the dose range tested. It is helpful to point out here that an alternative standard protocol of dividing the LOAEL, 50 µg/kg-d in Heinze (1999), by 10 to estimate a NOAEL of 5 µg/kg-d provides a very similar point of departure as achieved using the BMD approach, 6.4 µg/kg-d."

From the 6.4 µg/kg/day BMDL, OEHHA then computed the reference dose (RfD[22]) by dividing by an uncertainty factor (UF) of 1000= 6.4 x 10-6 mg/kg/day (= 0.0064 µg/kg/day). From the RfD, OEHHA then computes the "action level" based on a child's exposure during a swimming event[23]. Exposure assumptions included an ingestion rate (IR) of 0.05 L/hr; a duration of 5 hours; and a body weight of 30.25 kg (see Appendix A of OEHHA doc). The "action level" [24] arrived at in this fashion is **0.8 µg/L**.

2) The CA Voluntary Guidance document (SWRCB 2010) relies upon a 2005 Oregon risk assessment (see Appendix 7 of CA guidance document) for the recreational posting guideline level of **8 µg/L**.

3) The 2005 Oregon Recreational Guidance document (OHA 2005) arrives at **8 µg/L** by using the Fawell et al. (1994) and Fawell and James (1994)/WHO tolerable daily intake value (TDI) of 0.04 µg/kg/day as follows from Oregon Appendix A:

Concentration of toxin (µg/L) = $\frac{\text{TDI x BW}}{\text{IR}}$

Where,
TDI (tolerable daily intake) = 0.04 µg/kg/day
BW (body weight) = 20 kg
IR (ingestion rate) = 0.1 L

The TDI was developed by the World Health Organization based on repeated oral administration of microcystin-LR in mice and effects on the liver (Fawell and James, 1994). A body weight (BW) of 20 kg was used to represent a child. An ingestion rate (IR) was based on EPA guidance for incidental ingestion of surface waters, in which 0.05 L is accidentally ingested per one-hour event (Dang, 1996). For this guidance, it was assumed that a child would swim for up to two hours in a single day.

Using the parameters described above, the equation results in 8 µg/L of microcystin toxin. According to World Health Organization guidance, 8 µg/L would correspond to approximately 40,000 cells/mL if *Microcystis* were the dominant species (Chorus & Bartrum, 1999). *Planktothrix* was included in the additional guidance, since it has the potential to contain higher endocellular microcystin compared with *Microcystis* (Codd et al., 2005).

[22] The RfD represents the maximum dose to which people could be exposed without significant risk of adverse health effects.
[23] The exposure assessment was based on a child swimming because they are the group with the highest exposure in this scenario (i.e., this age group tends to swallow more water while swimming than other age groups). By basing exposure on the highest exposure group, other groups with less exposure are also covered.
[24] OEHHA defines an "action level" as: "*scientifically based health protective "action levels" that may be applied as needed, by local, regional, state or tribal entities throughout California, to reduce (or eliminate) algal toxin exposures"*

Note: the 0.04 µg/kg/day TDI is based on the NOAEL of 40 µg/kg/day from the Fawell et al. studies divided by a combined UF of 1000.

Both the above and the following Oregon documents cite to the WHO to state that 8 µg/L and 10 µg/L microcystin would correspond to approximately 40,000 cells/ml if *Microcystis* were the dominant species. However this is highly variable and Klamath River-specific data indicate that 8 µg/L is often exceeded at cell densities lower than 40,000 cells/ml (see above).

4) The newest 2012 Oregon document (see Appendix A of the document) arrives at 10 µg/L microcystin by using the Heinze LOAEL of 50 µg/kg/day and converting that to a TDI of 0.05 µg/kg/day by dividing by a UF of 1000. Then, computed as below, they arrive at a guideline value of **10 µg/L**:

$$\text{Concentration of toxin (µg/L)} = \frac{\text{TDI} \times \text{BW}}{\text{IR}}$$

where:

TDI (tolerable daily intake) = 0.05 µg/kg/day
BW (body weight) = 20 kg
IR (ingestion rate) = 0.1 L

The TDI was developed by the Oregon Public Health Division (OPHD) based on oral administration of microcystin-LR via drinking water in rats and effects on the liver (Heinze, 1999). A body weight (BW) of 20 kg was used to represent a child. An ingestion rate (IR) was based on EPA guidance for incidental ingestion of surface waters, in which 0.05 L is accidentally ingested per one-hour event (Dang, 1996). For this guidance, it was assumed that a child would swim for up to two hours in a single day.

Additional information on Oregon's derivation is found on pgs. 12-13 of Appendix B :

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

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

$$\text{ATDI} = \frac{\text{LOAEL}}{\text{UF}}$$

Where:
ATDI = Acute 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 intraspecies variability).

This recommended ATDI should be considered provisional and will be updated either to conform to federal standards or when additional toxicological information becomes available.

Additional Support for this ATDI
The TDI developed by WHO (0.04 µg/kg-day) based on the Fawell, et al. study (Fawell et al., 1999a) is very similar to the provisional acute value (0.05 µg/kg-day) proposed here. OEHHA's selection (CalEPA, 2012) of the Heinze study (Heinze, 1999) also supports OPHD's decision to use the same study. A chronic (18 month) mouse toxicity study of microcystin LR in drinking water identified a NOAEL of 3 µg/kg-day (Ueno et al., 1999), very similar to the estimated 5 µg/kg-day NOAEL based on the Heinze study OPHD used to develop this provisional ATDI.

Summary
Based on the ATDI calculated above, the guideline value for microcystin in recreational water bodies is **10 µg/L**.

The difference between this and the previous Oregon version (2005; and the CA Draft Voluntary Guidance which used the earlier OR version) is that Oregon went from using a TDI based on a **NOAEL** of 40 µg/kg/day resulting in a TDI of 0.04µg/kg/day (Fawell et al. and WHO) to using a TDI of 0.05 µg/kg/day based on a **LOAEL** of 50 µg/kg/day (Heinze 1999). This change in toxic endpoint used in deriving the TDI resulted in the guideline level changing from 8 µg/L to 10 µg/L. Also as noted above, while the recent Oregon document used an UF of 1000, the breakdown was 10 for LOAEL to NOAEL, 10 for interspecies, and 10 for intraspecies. The previous Oregon guideline using the Fawell/WHO NOAEL also used an UF of 1000 to compute the TDI, but the breakdown was 10 for less-than-lifetime study, 10 for interspecies, and 10 for intraspecies. OEHHA also applies a UF of 1000 to their BMDL, but their breakdown is 10 for interspecies, 10 for intraspecies, and 10 for incomplete toxicology profiles particularly with regard to cancer and effects in children (see p. 15 of the OEHHA doc). Although the recent Oregon document utilized a UF of 10 to account for not having a NOAEL, it is not clear whether they did this in order to be consistent with the starting point for the earlier iteration (in which they did utilize a NOAEL) or why they dropped the 10X UF for less-than-lifetime study[25].

Also note that Oregon used an available acute RfD (ARfD) for Saxitoxins, and a subchronic RfD for cylindrospermopsin to compute the guideline values for those toxins (see pgs. 16 and 17 of Appendix B in OHA 2012), possibly implying that the use of RfD's is the preferred approach when such information is available.

5) The Washington 2008 guidelines also utilized the Fawell et al. 1999/WHO tolerable daily intake value (TDI) of 0.04 µg/kg/day. However, unlike the earlier Oregon Risk assessment doc which arrived at 8 µg/L, WA arrives at **6 µg/L** by using a smaller body weight for a child, in this case 15 kg:

Washington Recreational Guidance Values: Microcystins

For Washington, DOH recommends the use of a recreational guidance value for microcystins (provisional) calculated as follows:

$$\text{Guidance value (µg/L)} = \frac{\text{TDI X BW}}{\text{IR}}, \text{ where}$$

TDI = 0.04 µg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d.

The tolerable daily intake (TDI) used in the above equation was developed by WHO based on microcystin-LR orally administered to mice, with observed effects on the liver. This limit accounts for chronic exposure to microcystin, including daily swimming, incidental ingestion, and inhalation through sinus passages. The resulting (provisional) recreational guidance value for Washington, as recommended by DOH, is 6 µg/L. This recommendation has been reviewed and accepted by DOH's Scientific Advisory Committee.

DOH's recommendation may change in the future if EPA's DRAFT risk reference dose of 0.006 µg/kg-day (derived for short-term and subchronic exposure durations) is adopted after review (USEPA 2006a). Because EPA's risk reference dose has not been adopted and because it is inconsistent with the WHO TDI, DOH's recommended recreational guidance value is provisional and may be updated as new information becomes available or if federal guidelines are developed.

[25] According to EPA (2002) the five standard UF's are 1) **Intraspecies** - the variation in sensitivity among the members of the human population; 2) **Interspecies** - the uncertainty in extrapolating animal data to humans; 3) **Subchronic-to-Chronic** - the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure; 4) **LOAEL-to-NOAEL** - the uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and 5) **Incomplete database** - the uncertainty associated with extrapolation when the database is incomplete. However, EPA (2002) recommends limiting the total UF applied for any particular chemical to no more than 3000 and avoiding the derivation of a reference value that involves application of the full 10-fold UF in four or more areas of extrapolation.

Note the WA reference to a Draft USEPA RfD of 0.006 µg/kg/day, a value similar to the OEHHA RfD of 0.0064 µg/kg/day. These values are essentially the same, except that OEHHA did not round as did EPA (OEHHA used the same studies and parameters as the earlier Draft USEPA document, which at the time of this writing is still in 'draft' form).

Summary and Discussion

Previous microcystin risk assessments to derive public health guideline values by CA, OR, and WA utilized the WHO TDI of 0.04 µg/kg/day to compute their public health guideline values. The WHO TDI was based on the NOAEL of 40 µg microcystin per kg body weight per day from the Fawell et al. (1999) study with an added uncertainty factor of 1000 (40/1000=0.04).

Both the 2012 Oregon guideline document and the 2012 OEHHA document utilize the Heinze (1999) study. As OEHHA notes on p. 14-15 there were several reasons why the Heinze study was utilized:

> "Two potential studies are available on which to base a short-term RfD: The Fawell [89] mouse study used in determining the WHO TDI [2] and the Heinze rat study [90]. WHO did not have the benefit of the Heinze study since it was published after their evaluation. Both the Fawell [89] and Heinze [90] studies found liver toxicity and used overlapping doses. The study on mice by Fawell identified a No Observable Adverse Effect Level (NOAEL) of 40 micrograms per kilogram of body weight per day (µg/kg-d) and a Lowest Observable Adverse Effect Level (LOAEL) of 200 µg/kg-d, which was the next highest dose level. The study on rats by Heinze used lower doses and identified a LOAEL of 50 µg/kg-d. OEHHA chose the Heinze study as the basis of the RfD because it evaluated more endpoints, utilized a better experimental design, showed greater target organ specificity (intrahepatic hemorrhage) in the histopathological analysis, and showed a clear dose-response trend. The rats of the Heinze study showed a greater sensitivity to microcystin-LR than the mice of the Fawell study."

However, while Oregon utilizes the Heinze LOAEL divided by an UF of 1000 to compute their guideline of 10 µg/L microcystin; OEHHA uses Heinze to estimate the BMDL and then divides that by an UF of 1000 to compute their RfD, which is then used to compute the "action level" of 0.8 µg/L. Because the BMDL[26] is more similar to a NOAEL than a LOAEL, in effect the OEHHA study derives something more equivalent to the WHO NOAEL utilized to compute the TDI (which again was the Fawell NOAEL/1000). In other words, the WHO TDI computed from the Fawell NOAEL/1000 and the OEHHA RfD computed from the Heinz BMDL/1000 are similar in that they both used a dose that is estimated to cause little or no effects and that both applied uncertainty factors of 1000.

Aside from whether the WHO TDI (0.04 µg/kg/day), the Oregon 2012 TDI (0.05 µg/kg/day), or the OEHHA RfD (0.0064 µg/kg/day) is used, further differences in the computed level for issuing advisories (Oregon) or what OEHHA calls an action level are due to the assumptions used (e.g., body weight and ingestion rate) for daily toxin exposure. For example using the equation from above (also see above for exposure assumptions):

[26] The BMDL essentially estimates the point at which there are very low to no effects, and the NOAEL is the level at which no adverse effects are observed.

Microcystin Conc. (µg/L) $= \frac{TDI\ or\ RfD\ x\ BW}{IR}$

Where: TDI or RfD is in units of µg/kg/day; BW is in units of kg; and IR is in units of L/day

For OEHHA: $\frac{0.0064\ x\ 30.25}{0.05\frac{L}{hr} x\ 5hr} = 0.8$ µg/L

Since OEHHA exposure assumptions are based on data and recommendations by USEPA, it seems prudent that even if the Oregon TDI based on the LOAEL is used in the equation, that California guidelines be based on the OEHHA recommended exposure assumptions.

Thus, using the OEHHA exposure assumptions with Oregon's TDI from the LOAEL:

Microcystin Conc. (µg/L) = $\frac{0.05\ x\ 30.25}{0.05\frac{L}{hr} x\ 5hr} = 6.0$

The original microcystin guideline of 10 µg/L using Oregon exposure assumptions then decreases to 6 µg/L using the OEHHA exposure assumptions.

Since both Oregon and OEHHA deem Heinze to be a better study, aside from the exposure assumptions, the main divergence between Oregon and OEHHA is whether the LOAEL or BMDL (which may approximate the NOAEL) is used as the starting point. Given that the previous work by WHO and Oregon utilized a NOAEL to determine the TDI, it is not clear whether a similarly conservative value[27], in this case the BMDL and subsequent RfD computed from the Heinze study should be used, at least as a starting point, to determine the guideline level at which an advisory is issued (Oregon's terminology) or "action level" is determined (OEHHA terminology).

In addition, it seems apparent that all entities were attempting to begin calculation (by dividing by UF's) of the TDI or RfD from a NOAEL or something approximating it (as opposed to beginning with the LOAEL); whether as determined directly from the dose-response curves (e.g., WHO/Fawell et al. 1994), by calculation of a BMDL (e.g., OEHHA), or by converting the LOAEL to a NOAEL by dividing by 10 (e.g., Oregon). In the case of Oregon, it is not clear how the decision was made to exclude the previously used 'less than lifetime' UF when the LOAEL to NOAEL UF was added. It should be noted that the original WHO TDI calculation started with the NOAEL and then used UF's of 10x for inter-species, 10x for intra-species, but also included a similar 10x UF for carcinogenicity and lack of data on chronic toxicity (less than lifetime).

Following is a summary in tabular form with highlighted cells denoting the values used by each entity to compute either the TDI or RfD:

[27] Oregon derived a microcystin concentration that was 1,000 times lower than the concentration shown to cause microscopic liver lesions in 6 of 10 rats. OEHHA derived a microcystin concentration that was 1,000 times lower than the concentration that is estimated to have essentially no effects on the rats.

Entity	Study	LOAEL (µg/kg/day)	NOAEL (µg/kg/day)	BMDL = 95% LCL on the 10% Response Rate or BMD (µg/kg/day)	Uncertainty Factor	TDI-Oregon or RfD-OEHHA (µg/kg/day)	Ingestion Rate Assumption (L/hr)	Water Contact Time Assumption (hrs)	Child Body Weight Assumption (kg)	Microcystin Criteria (OR and WA) or Action Level (CA) (µg/L)[e]
CA OEHHA	Heinze 1999	50	5[a]	6.4	1000[b]	0.0064	0.05	5	30.25	0.8
Oregon 2012	Heinze 1999	50	n/a	n/a	1000[c]	0.05	0.05	2	20.0	10.0
Oregon 2012 using OEHHA exposure assumptions	Heinze 1999	50	n/a	n/a	1000[c]	0.05	0.05	5	30.25	6.0
WHO (1999)	Fawell et al. 1994	200	40	n/a	1000[d]	0.04				
CA Voluntary Guidance & Older OR	Fawell et al. 1994	200	40	n/a	1000[d]	0.04	0.05	2	20.0	8.0
WA	Fawell et al. 1994	200	40	n/a	1000[d]	0.04	0.05	2	15.0	6.0

[a] Estimated by OEHHA by dividing the LOAEL by a factor of 10.

[b] 10x for inter-species differences; 10x for intra-species differences; 10x for incomplete toxicology profiles (cancer and effects in children).

[c] 10x for LOAEL to NOAEL; 10x for inter-species differences; 10x for intra-species differences.

[d] 10x for inter-species differences; 10x for intra-species differences; 10x for limitations in the database (chronic toxicity and carcinogenicity).

[e] Microcystin Conc. (µg/L) = $\frac{TDI(RfD) \; x \; BW}{}$

To reiterate, the Table above shows that the main divergence between the values is being caused by using different toxic endpoints for calculating the TDI - specifically the difference of using LOAELs versus NOAEL or BMDL. The various criteria or action level values differ due to the different toxicological studies relied upon; specifically the CA OEHHA BMDL (an extrapolated value, not a tested value) is approximately 7 times lower than the older Fawell study NOAEL (40 µg/kg/day). Additionally, the older Fawell study NOAEL(40 µg/kg/day) is close in value to the newer Heinze study LOAEL (50 µg/kg/day). However, the estimated Heinze NOAEL (5 µg/kg/day) is also similar to the BMDL, and Oregon includes an UF of 10x to account for not having a NOAEL for the Heinz study.

Corresponding actions taken in response to exceeding these different Action Levels should reflect the toxic endpoint they are based on. Specifically, the 2012 Oregon value is related to a level having an impact (liver lesions); however, the CA OEHHA value was developed from an RfD that "represents the maximum dose to which people could be exposed without significant risk of adverse health effects". So where Oregon's action is to post water bodies when criteria are exceeded; CA needs to consider what action to implement *when waters reach the maximum level where people could be exposed without significant risk of adverse health effects*.

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OHA 2005 in its entirety:

Public Health Advisory Guidance for Toxigenic Cyanobacteria in Recreational Waters

Currently, several water bodies in Oregon are monitored for toxigenic cyanobacteria. In past years, the decision-making process for issuing and lifting advisories varied with the managing jurisdiction of that water body. The intent of this document is to provide statewide public health guidelines for issuing and lifting advisories in recreational waters when toxigenic cyanobacteria are detected. While it is hoped these recommendations are applied consistently across Oregon, site-specific issues and flexibility in the decision-making process is emphasized. These guidelines are intended for recreational exposures only and not for water bodies that serve as drinking water sources. In addition, these guidelines are recommendations based on the best available information and subject to change if needed or as more information becomes available.

Toxigenic Cyanobacteria

Cyanobacteria, also known as blue-green algae, are commonly found in many freshwater systems across the world. The species of concern for these guidelines are referred to as toxigenic species, since they have the potential to produce toxins. The primary target organs for cyanotoxins are the liver and nervous system, although other health effects are possible.

Currently, at least 46 species of cyanobacteria have been shown to be toxic to vertebrates (Chorus & Bartrum, 1999). Some of the more common toxigenic genera include *Microcystis*, *Anabaena*, *Aphanizomenon*, *Lyngbya*, *Nodularia*, *Planktothrix*, *Nostoc* and *Cylindrospermopsis*. The cyanotoxins that have been detected in non-marine waters of Oregon include microcystin, anatoxin-a and cylindrospermopsin. While cyanobacteria can produce other toxins, the focus of this section will be on microcystin and anatoxin-a, the most commonly detected cyanotoxins in Oregon lakes. It should be noted that cyanobacteria likely produce toxins that have not been characterized. A recent example is the discovery of a neurotoxic amino acid that can be produced by the majority of cyanobacteria (Cox et al., 2005).

Microcystin

Microcystins are the most commonly detected cyanotoxin across the globe (Chorus and Bartrum, 1999). Cyanobacteria that are known to produce microcystins include *Microcystis, Planktothrix, Oscillatoria, Nostoc, Anabaena, Anabaenopsis and Hapalosiphon*. Microcystins are cyclic heptapeptides with about 60 known structural variants (Rinehart et al., 1994). These structural 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 use a carrier similar to the bile acid carrier of liver cells. Exposure to microcystin has the potential to cause acute and chronic injury, depending on the dose and duration of 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 that were initiated with a known carcinogen (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 days (Short and Edwards, 1990). Symptoms reported from British Military recruits exposed to a bloom of *M. aeruginosa* during an exercise in a reservoir included abdominal pain, vomiting, diarrhea, sore throat, blistering of the mouth and pneumonia (Turner et al., 1990).

A Tolerable Daily Intake (TDI) was calculated for microcystin-LR, since this variant has sufficient information to derive a guideline value and is thought to be one of the most toxic variants. A TDI is a level of exposure below which it is thought that no adverse health effects will occur. It is important to note that simply exceeding a TDI does not imply that a health effect is likely. Rather, the duration of exposure and concentration of toxin will be major determinants of toxicity. The basis for the TDI was a 13-week mouse study with observed liver changes (Fawell et al., 1994). The no observed adverse effect level (NOAEL), which was the basis for determining a guidance value, was 40µg microcystin per kg body weight per day. To calculate a TDI, the NOAEL was divided by a series of uncertainty factors to include potential for intraspecies variation (factor of 10), interspecies variation (factor of 10) and for a less-than-lifetime study (factor of 10). The equation is:

$$\text{TDI} = \frac{40\ \mu g/kg \bullet day^{-1}}{1000} = 0.04\ \mu g \text{ microcystin-LR per kg body weight per day}$$

The TDI is instrumental in determining guidance for taxa that are known to produce microcystins at high intracellular concentrations, such as *Microcystis* or *Planktothrix.* This process is described in Appendix A.

Anatoxin-a

Anatoxin-a is an alkaloid neurotoxin that is produced by some strains of *Anabaena, Aphanizomenon* and *Oscillatoria* (Chorus & Bartrum 1999). Anatoxin-a mimics the neurotransmitter acetylcholine, binds to nicotinic acetylcholine receptors and cannot be degraded by the enzyme acetylcholinesterase. The molecular activity of anatoxin-a leads to over stimulation of muscle cells and possibly paralysis followed by asphyxiation (Carmichael 1997). In addition to anatoxin-a, anatoxin-a(s) and homoanatoxin have been identified from cyanobacteria and vary in their toxicity and mode of action.

The acute toxic properties of anatoxin-a are obvious, since it affects the nervous system. Available data indicate that it is unlikely to cause chronic toxicity from limited exposure (Fawell & James 1994). At this time, the database is insufficient for a derivation of a TDI as human exposure information and suitable animal tests are lacking.

Exposures Pathways

The primary exposure pathway of concern for exposure to cyanotoxins is through ingestion of water. Dermal effects are possible from the lipopolysaccharides found on cell surfaces, however the 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 pathways. Incidental ingestion is more likely in recreational waters, especially in turbid or discolored lakes. The risk of incidental ingestion is particularly high for children playing in near-shore areas where scums tend to accumulate. Exposure levels can be broadly defined as high, moderate and low based on recreational activity (Table 1).

Table 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

A possible scenario for the intentional ingestion of recreational water that should be considered is the use of lake water for drinking or cooking purposes by campers and hikers. It is possible that some campers or hikers have the mistaken belief that boiling, filtering or treating contaminated water with camping equipment will make it potable. This scenario should be addressed in informational and advisory signs.

At this time, there is insufficient information to determine the risk of consuming fish caught in waters with toxigenic cyanobacteria. Studies have shown that toxins mainly accumulate in the liver and viscera of fish, although microcystin has been detected in the fillet (Vasconcelos, 1999; de Magalhães et al., 2001). At a minimum, the organs and skin should be removed and discarded prior to cooking fillets. In addition, shellfish have been shown to accumulate cyanotoxins in edible tissue (Vasconcelos, 1999). It is recommended that people call the Department of Human Services for more information on fish consumption while advisories are in effect.

Issuing Advisories

In 2004 and previous years, lakes were posted when toxigenic cell densities exceeded 15,000 cells/mL (corresponding to an Alert Level III using World Health Organization recommendations). The guidance below recommends that agencies not use 15,000 cells/mL as an absolute criterion for posting advisories at recreational access points. The risk to recreational users at this cell density is considered low and includes symptoms such as skin irritation and gastrointestinal disorders, which are though to be related to lipopolysaccharide endotoxins found on cell walls. In a recent study, acute skin irritant effects were tested over a range of cell densities (< 5000 cells/mL to > 200,000 cells/mL) after application of cyanobacterial extracts (Pilotto, 2004). Genera tested included *Anabaena*, *Microcystis*, *Cylindrospermopsis* and *Nodularia*. Approximately 15% of the people reacted to the extracts, with mild, self-limiting reactions. Furthermore, no dose-response relationship was established. The absence of a dose-response relationship, and therefore a threshold, makes it difficult to recommend quantitative guidance. Consequently, the focus of advisory postings is on the risk posed by cyanotoxins and the potential for systemic effects.

Despite the lack of quantitative guidance to address the potential for mild reactions to cyanobacteria, such as skin irritation, DHS recommends that posters and pamphlets be available to advise the public about these possibilities. Information should be posted and visible at kiosks, bulletin boards and other suitable locations that describe these effects and symptoms. Additional suggested information for these postings or pamphlets include:

- advice that if symptoms persist or become more severe over time, to contact their medical provider
- notice that not all waters can be monitored all the time and scummy, turbid or discolored waters should be avoided
- notice that algae cells trapped beneath clothing may be more likely to cause skin reactions and washing with clean water is recommended
- warning that people with nasal-bronchial allergies may be more susceptible to skin irritation from cyanobacteria
- warning that children, immunocompromised individuals and the elderly are more susceptible to gastrointestinal disturbances

Figure 1 depicts a flowchart of guidelines to assist in deciding whether to post or not post a waterbody. The issuance of advisories is based solely on cell density determinations and not dependent upon the analysis of toxins. However, the analysis of toxin data is recommended to better understand the systems being monitored, the potential health implications and to document historical trends for future advisories.

If *Microcystis* or *Planktothrix* is not the dominant species in a sample, DHS recommends advisories be posted if cell densities of total toxigenic cyanobacteria equal or exceed 100,000 cells/mL, or if scums containing toxigenic cyanobacteria are observed. At 100,000 cells/mL, the World Health Organization lists a moderate probability of adverse health effects, based in part on the ability of cyanotoxins to reach levels of concern. As the cell density increases, the

Figure 1. Proposed Guidance for Recreational Contact with Cyanobacteria

Hazard Identification

What species of toxigenic cyanobacteria are present?

What is the density of cells per mL of toxigenic species in the water?

Posting Decisions:

Part A: Is scum visible and associated with toxigenic species?

No: Go to part B

Yes: Post

Part B: Is *Microcystis* or *Planktothrix* present?

No:		Yes:	
Is the sum of the potentially toxigenic* taxa > 100,000 cells/mL?		Is the cell density of *Microcystis* or *Planktothrix* > 40,000 cells/mL?	
Yes: Post	No: Do not post	Yes: Post	No: Do not post

*Potentially toxigenic taxa that have been detected in Oregon include *Anabaena, Microcystis, Planktothrix, Nostoc, Coelosphaerium, Anabaenopsis, Aphanizomenon, Gloeotrichia* and *Oscillatoria.* Additional taxa that are known to be potentially toxigenic may be added to this list.

potential for frequently occurring cyanobacteria to form scums may increase toxin production by 1000x in a few hours (Chorus and Bartrum,1999). Toxigenic genera that are common scum producers include *Microcystis, Anabaena, Anabaenopsis, Planktothrix and Aphanizomenon* (Codd et al., 2005). A lower guideline of 40,000 cells/mL was recommended for issuing

advisories based on cell densities that are dominated by *Microcystis* and *Planktothrix*. This lower guideline is based on the premise that these two genera are more likely to produce microcystin toxin compared to other genera, such as *Anabaena* (Codd et al., 2005; Chorus and Bartrum, 1999) and the observation that almost all *Microcystis* strains are toxic (Carmichael, 1995). To derive the guideline of 40,000 cells/ml, a risk assessment approach was employed based on recreational exposure to microcystin toxin to a child (Appendix A).

Currently, no TDI or reference dose has been established for anatoxin-a, prohibiting the quantitative approach that was used for microcystin. Detection of anatoxin-a or any other cyanotoxin in recreational waters should be handled on a case-by-case basis, involving expert consultation for public health and lake access decisions.

Lifting advisories

Cyanotoxins, if produced, are found within the cell during most of a bloom event. However, toxin may be released into the water when the cells die and lyse. The released toxin will dilute and eventually degrade over time. However, the risk of exposure to dissolved toxin immediately following the peak of a bloom must be addressed since cyanotoxins have been detected in the water phase as a result of extracellular release, even though the producer cells (i.e. cell density) are absent or found in low numbers (Lawton, 1994). An additional risk factor is that the water will appear more suitable for recreational activities as clarity increases, thus elevating the potential for exposure during this period.

It is recommended that an advisory be lifted after a waiting period of **two** weeks once the cell density of potentially toxigenic blue-green algae falls below the thresholds established in Part B of the Guidelines (Figure 1) and with sufficient evidence that the bloom is continuing to decline. Evidence of a declining bloom can include decreasing cell density of potentially toxigenic cyanobacteria and increasing lake clarity.

An advisory may be lifted **one** week after the cell density of potentially toxigenic blue-green algae falls below the thresholds established in Part B of the Guidelines (Figure 1) if toxin analysis indicates that microcystin is below 8 ug/L for species capable of producing microcystin and anatoxin-a is below 3 ug/L detected for species capable of producing anatoxin-a. It is recommended that if the dominant species of an advisory is known in the scientific literature to produce anatoxin-a and microcystin, that both toxins be tested prior to lifting an advisory before the two-week waiting period.

The advisory should remain in place until a final quantitative sample confirms the decreasing trend of potentially toxigenic blue-green algae and restrictions should remain in place whenever scums are visible. In some situations, there may be reason to prolong the advisory beyond the recommended waiting period. This may result from reported illnesses associated with recreational contact, the persistence of toxin, historic concerns at a particular water body (such as the Diamond Lake 2001 event), or other factors. Furthermore, it is likely that certain water bodies will have site-specific issues that require consultation among stakeholders to determine suitable actions to address an advisory.

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Appendix A. Risk Assessment for deriving quantitative guidance for blooms dominated by *Microcystis* or *Planktothrix*

A focused risk assessment was conducted to characterize the risk associated with swimming in waters that are dominated by *Microcystis* or *Planktothrix* cyanobacteria.

The equation and parameters are described below:

$$\text{Concentration of toxin } (\mu g/L) = \frac{\text{TDI x BW}}{\text{IR}}$$

where,

TDI (tolerable daily intake) = 0.04 µg/kg/day
BW (body weight) = 20 kg IR
(ingestion rate) = 0.1 L

The TDI was developed by the World Health Organization based on repeated oral administration of microcystin-LR in mice and effects on the liver (Fawell and James, 1994). A body weight (BW) of 20 kg was used to represent a child. An ingestion rate (IR) was based on EPA guidance for incidental ingestion of surface waters, in which 0.05 L is accidentally ingested per one-hour event (Dang, 1996). For this guidance, it was assumed that a child would swim for up to two hours in a single day.

Using the parameters described above, the equation results in 8 µg/L of microcystin toxin. According to World Health Organization guidance, 8 µg/L would correspond to approximately 40,000 cells/mL if *Microcystis* were the dominant species (Chorus & Bartrum, 1999). *Planktothrix* was included in the additional guidance, since it has the potential to contain higher endocellular microcystin compared with *Microcystis* (Codd et al., 2005).