Technical Memorandum

Microcystin Bioaccumulation in Klamath River Freshwater Mussel Tissue: 2009 Results

PREPARED BY

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INTRODUCTION

Copco and Iron Gate Reservoirs (the lowermost projects of PacifiCorp’s Klamath Hydropower Project-- KHP) experienced extensive blooms of toxigenic *Microcystis aeruginosa* (MSAE) from 2004-2009 (Kann and Corum 2009; 2010; Jacoby and Kann 2007). These blooms were associated with high levels of the cyanotoxin microcystin, a potent hepatotoxin capable of causing chronic liver damage and acting as a tumor promoter (Carmichael 1995; Chorus et al. 1999; Chorus 2001).

The results of the 2005-2009 sampling program demonstrated widespread and high abundance of toxigenic MSAE blooms in Copco and Iron Gate reservoirs and in the Klamath River downstream, exceeding World Health Organization Moderate Probability of Adverse Health Effect Levels for both cell density and toxin by 10 to over 1000 times.

In addition, bioaccumulation studies undertaken in 2007 and 2008 showed accumulation of microcystin toxin in muscle and/or liver tissues of yellow perch, hatchery salmon, and freshwater mussels (Mekebri et al. 2009; Kann 2008; Kanz 2008). Microcystin levels in biota exceeded public health threshold values for safe consumption (Kann 2008; OEHHA 2008). The following report summarizes the Karuk Tribe Department of Natural Resources and Yurok Tribe Environmental Program 2009 sampling program to evaluate microcystin bioaccumulation in freshwater mussels in the Klamath River.

METHODS

Station Location and Frequency

During the 2009 sampling season freshwater mussels were collected by the Karuk and Yurok Tribes at a variety of locations on the Klamath River (Figure 1 and Figure 2). Karuk Tribe stations included the Klamath River at the I5 Bridge (IB), Brown Bear river access (BB), Seiad Valley (SV), Happy Camp (HC), and Orleans (OR). The primary Yurok Tribe station was located on the Klamath River just upstream of Starwein Riffle (KA), as well as one sample collected on the Trinity River (TR) just upstream from the Klamath River confluence (Figure 2). The primary stations were generally sampled monthly from July through October, but TR was sampled only once in September, and BB was sampled one additional time in December (Figure 3).

Ambient concentrations for microcystin were collected in conjunction with the freshwater mussel samples, as well as at additional locations to evaluate bioaccumulation trends relative to ambient concentrations (Figure 3). Ambient data from the Karuk Tribe’s 2009 public health monitoring program (Kann and Corum 2010) was also evaluated with respect to bioaccumulation trends. Ambient data for the Yurok Tribe station TG, which is close to the KA mussel collection station, were available on dates when mussels were collected and is also included in below analyses.
Figure 1. Location of Karuk Tribe freshwater mussel sampling locations, 2009.

Figure 2. Location of Yurok Tribe freshwater mussel sampling locations, 2009. Note that mussel’s were collected at stations KA and TR.
Sample Collection and Lab Analysis

Klamath River freshwater mussel beds were sampled either via snorkeling or wading (if depth permitted) at the above locations (Figure 4). In general 5 to 6 individual mussels (whole *Gonidea angulata*) from each location were selected from the bed, wrapped in foil, and then frozen prior to shipment to the California Department of Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova, CA. Extraction of tissue samples included homogenization and sonification prior to using the LC-ESI-MS-MS method to determine the concentration of major microcystin congeners (Mekebri et al. 2009). Congeners analyzed included: MCY-RR, MCY-Desmethyl-RR (MCY-RRDM), MCY-LR, MCY-Desmethyl-LR (MCY-LRDM), MCY-YR, MCY-LA, MCY-LW, MCY-LF, and MCY-LY. In addition, the neurotoxins anatoxin-a, domoic acid, and okadaic acid were also analyzed.

Samples for ambient microcystin concentration were collected using the standard operating procedure (SOP) developed by the Klamath Blue-Green Algae Working Group (see Kann and Corum 2010 for details). These were analyzed as per the liquid sample extraction method in Mekebri et al. (2009). Methodology for additional ambient samples that were collected as part of the Karuk Tribe’s public health monitoring program for cyanobacteria is outlined in Kann and Corum 2010.
Laboratory quality assurance consisting of split samples for both tissue and ambient microcystin toxin concentrations were also provided (Figure 3; denoted with a “D” after the station name). These data are contained in Appendix I, and generally showed good agreement between tissue duplicates (Figure 5).

Figure 5. Comparison of duplicate mussel samples for stations in the Klamath River system collected in 2007 and 2009.
Comparison to Public Health Threshold Values

The following comparison of Klamath River microcystin tissue concentrations to public health guideline values is based on a recent comprehensive review of cyanobacterial toxin accumulation by Ibelings and Chorus (2007). Table 2 from Ibelings and Chorus (2007) entitled “Tolerable doses to microcystin-LR in relation to frequency and duration of exposure” is reproduced here:

<table>
<thead>
<tr>
<th>Temporal pattern of exposure and ensuing Tolerable Intake (TI)</th>
<th>Assumptions</th>
<th>Tolerable Intake per kg</th>
<th>Tolerable Intake for a 10 kg child</th>
<th>Tolerable Intake for a 75 kg adult</th>
<th>Guideline value for food (µg kg⁻¹) AF = 1</th>
<th>AF = 0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute TI</td>
<td>NOAEL¹ of 250 µg/kg and day, extrapolation factors of 100</td>
<td>2.5 µg per kg and single exposure</td>
<td>25 µg per single exposure</td>
<td>190 µg per single exposure</td>
<td>Adult: 1900, Child: 250</td>
<td>Adult: 380, Child: 50</td>
</tr>
<tr>
<td>Seasonal TDI</td>
<td>NOAEL of 0.4 µg/kg and day, extrapolation factors of 100 (Chorus and Bartram, 1999, adapted)</td>
<td>0.4 µg per kg and day</td>
<td>4 µg per day</td>
<td>30 µg per day</td>
<td>Adult: 300, Child: 40</td>
<td>Adult: 60, Child: 8</td>
</tr>
<tr>
<td>Lifetime TDI</td>
<td>NOAEL of 0.4 µg/kg and day, extrapolation factors of 100 and uncertainty factor of 10 (Chorus and Bartram, 1999)</td>
<td>0.04 µg per kg and day</td>
<td>0.4 µg per day</td>
<td>3 µg per day</td>
<td>Adult: 30, Child: 4.0²</td>
<td>Adult: 6, Child: 0.8²</td>
</tr>
</tbody>
</table>

Tolerable doses in seafood related to the frequency and duration of the exposure. A distinction is made between intake by small children and adults, and a further distinction between an Allocation Factor (AF) of 1 (toxins present in food only) and – following the derivation of the provisional WHO GV for Drinking-water – an AF of 0.2 (80% of the dose is taken in—mainly—via drinking water, only 20% via food). For calculating guideline values, following eq. (2) in Section 3 a consumption (C) of 100 g fish (per day) is assumed. Acute TI: single exposure event (e.g. week-end fishing trip). Seasonal TDI: ongoing, “daily” exposure for several weeks during the cyanobacterial season. Lifetime TDI: ongoing “daily” exposure for many months in settings where microcystin-producing cyanobacteria proliferate perennially. ¹NOAEL= no observed adverse effect level. ²Original values in Ibelings and Chorus contained a typo and were listed incorrectly as 0.4 and 0.08 µg/kg; correct values are as shown above.

Previous analyses (Kann 2008) evaluated three of the congeners (-LR, LR-DM, and –LA) with respect to the guideline values derived by Ibelings and Chorus (2007) that are based on toxicity work for microcystin-LR. However, because this approach likely underestimates toxicity due to the exclusion of several of the congeners, similar to the California Office of Environmental Health Hazard Assessment (OEHHA), the below comparisons utilize a more conservative approach with respect to public health that includes the sum of all microcystin congeners. For the following comparisons to Ibelings and Chorus (2007) it is assumed that the only exposure is through ingestion; therefore guideline values were evaluated for Allocation Factor=1 (see reproduced Table 2 above for AF description as well as assumptions regarding frequency and quantity of tissue consumed). In addition, OEHHA calculated the maximum number of 8-oz meals per month at varying microcystin levels in tissue (see Appendix II below); these values are equivalent to the Seasonal TDI guidance value as shown by Ibelings and Chorus in Table 2 above. For example the recommended OEHHA microcystin level above which a child should not consume even one meal per month is 40 µg/L, and is the same level as the Ibelings and Chorus (2007) Seasonal TDI guideline value for a child as shown above.
Trends in Tissue and Ambient Toxin Concentration

The neurotoxins anatoxin-a, domoic acid, and okadaic acid were not detected in any of the samples (Appendix I) and will not be discussed further. Of all microcystin congeners measured, only four were detected: MCY-RR, MCY-LR, MCY-LA, and MCY-LRDM (Appendix I). These showed a distinct seasonal pattern in freshwater mussel tissue, with most stations in July dominated primarily by MCY-LR and MCY-LA, and secondarily by MCY-LRDM (Figure 6). Aside from the Yurok station KA, which increased in August from non-detects in July, concentration at the remaining upstream stations decreased in August, with MCY-LRDM not detected for the remainder of the season (Figure 6). The concentration of MCY-LA increased again in September, but MCY-LR remained proportionally lower, especially with respect to July. In addition, MCY-RR was first detected in September at several stations (Figure 6). The overall seasonal pattern of the 3 most dominant congeners over all stations shows July domination by both MCY-LR and MCY-LA and although MCY-LRDM was present in July it was not detected for the remainder of the season; both MCY-LR and MCY-LA declined in August, but only MCY-LA rebounded in September and October; only MCY-LA was detected in a final set of samples taken in December at station BB (Figure 7).

Figure 6. Concentration of microcystin-LR, LA, LR-DM, and RR (ng/g or ppb) in whole freshwater mussels collected from stations (IB, BB, SV, HC, OR, KA) in the Klamath River system in 2009. Horizontal lines denote the Lifetime, Seasonal, and Acute Public health threshold levels.
A comparison of tissue concentration data relative to ambient concentrations reveals that both MCY-LR and MCY-LA were detected in both media, while MCY-RR and MCY-LRDM were detected in tissue data but they were not detected in water samples (Figure 8). In addition, the frequency of MCY-LR detection in tissue was greater than that for water. The lack of detection or low frequency of detection in water relative to tissue samples indicates that although these congeners may have been below detection in water samples, through bioaccumulation mechanisms were then found in proportionally greater frequency (as well as higher concentration) in freshwater mussel tissue.

Corresponding to the August decline in tissue bioaccumulation, the seasonal trend in ambient data also indicates a decline (most noticeable at station BB) in the total microcystin concentration during some of the August dates (Figure 9). A decline in August Microcystis aeruginosa cell density and microcystin concentration was also observed in the upstream reservoir system during August of 2009 (Kann and Corum 2010).

The monthly pattern in total microcystin concentration in freshwater mussel tissue indicates relatively high values in July, September, and October, with highest overall values occurring in September and October (Figure 10a). Microcystin was still detected in mussels from the December sample, with several values continuing to exceed the Lifetime TDI for a child (Figure 10a).
Figure 8. Concentration of microcystin congeners (ng/g or ppb) in freshwater mussel samples and ambient (water) samples from the Klamath River system in 2009.

Figure 9. Ambient concentration of total microcystin samples collected from the Klamath River system in 2009.
Figure 10. Concentration by month (a) and location (b) of the sum of microcystin-RR, RR-DM, LR, LR-DM, YR, LA, LW, LF and LY in whole freshwater mussels collected from the Klamath River system in 2009 July to October. Stations ordered longitudinally left (upstream) to right (downstream).
A comparison among stations reveals that all stations showed some level of microcystin bioaccumulation, with no clear longitudinal pattern in the median or upper quartile values (Figure 10b). However, there was some indication of a longitudinal pattern in the lower quartile values, which aside from the I5 Bridge (IB) decreased downstream. Seiad Valley (SV) tended to show a lower overall distribution, and the highest upper quartile value was observed at Orleans (OR). Although the farthest downstream station, KA, showed the lowest lower quartile value, there were still numerous occurrences of total microcystin that exceeded the Acute TDI value for a child (Figure 10b). No microcystin was detected in mussels sampled on September 14th from the Trinity River (Appendix I: station TR).

Total microcystin values in ambient water were above the public health guideline values for posting on several occasions at stations BB, SV, and HC, but in general the majority of samples were below the 8 µg/L posting level (Figure 11). An overall comparison of ambient data to the tissue data reveals that despite relatively low ambient concentrations (Figure 11: median microcystin values were often less than 1 µg/L), substantial microcystin bioaccumulation occurred at all stations (Figure 10b). This was especially true for the most downstream station, KA, where numerous non-detects and low microcystin values were observed (stations KA and TG in Figure 11), yet levels found in freshwater mussel tissue were above the various public health thresholds (Figure 10b).

Figure 11. Ambient concentration of total microcystin samples collected from stations in the Klamath River system in 2009.
Comparison to Bioaccumulation in 2007 Freshwater Mussel Samples

Microcystin bioaccumulation in freshwater mussels was evaluated on two dates in 2007; one in July and one in November (Kann 2008). With the exception of MCY-RR which was present in July of 2007 but not 2009, other congeners were similar between the two years during July (the only time period for which samples overlapped in 2007 and 2009); with MC-LR, MCY-LA and MCY-LRDM all present in both years (Figure 12). It should be noted that although MCY-RR was not detected in July of 2009 it was detected in September of 2009. Comparison of the total microcystin concentration between the two years shows elevated bioaccumulation in July for both years, but by November of 2007 no microcystin was detected in the mussel tissue (indicating depuration), while microcystin continued to be detected in December of 2009 (Figure 13).

![Figure 12. Concentration of Total Microcystin from samples collected in July of 2007 and 2009 from the Klamath River System. Note: Positive concentrations of Microcystin RR found beginning in September 2009.](image-url)
Comparison to Public Health Threshold Values

Concentration of the three most prevalent microcystin congeners found in Klamath River freshwater mussel tissue shows that MCY-LR and MCY-LA levels exceeded all three guideline TDI levels for children (Lifetime, Seasonal, and Acute) at varying times in July, September and October, and that the concentration of MCY-LRDM often exceeded the Lifetime TDI guideline level when it was present in July (Figure 7). In addition, MCY-RR remained below the Lifetime TDI when it was present in September (Figure 6).

Temporal and spatial summaries of the total tissue microcystin concentration (the sum of all congeners) show that ingestion of freshwater mussels in the Klamath River system would result in microcystin doses that exceed various public health thresholds for safe consumption (Figures 14 and 15). This is especially true for children, where in the months of July, September, and October, the Acute Tolerable Intake (TI) dose was exceeded by up to ~4 times (Figure 14a). This means that even one meal of freshwater mussels for a child would exceed what is considered to be a safe level (OEHHA 2008). Exceedance of the Acute TI was shown to occur...
Figure 14. Exceedance of Child Lifetime, Seasonal, and Acute TDI for the sum of microcystin-RR, RR-DM, LR, LR-DM, YR, LA, LW, LF and LY in whole freshwater mussels collected from the Klamath River system in 2009 July to October; by month (a) and location (b). TDI values are as described in Ibelings and Chorus (2007; Table 2 reproduced above).
Figure 15. Exceedance of Adult Lifetime, Seasonal, and Acute TDI for the sum of microcystin-RR, RR-DM, LR, LR-DM, YR, LA, LW, LF and LY in whole freshwater mussels collected from the Klamath River system in 2009 July to October; by month (a) and location (b). TDI values are as described in Ibelings and Chorus (2007; Table 2 reproduced above).
at all stations, including the most downstream station, KA (Figure 14b). Total microcystin levels in freshwater mussel tissue consistently exceeded the Lifetime TDI guideline for children by 100’s of times, and the Seasonal TDI by over 10 times (Figure 14). In addition, although no exceedances of Acute TI levels for adults were observed, numerous exceedances of the adult Seasonal and Lifetime TDI levels were observed during 2009 (Figure 15). Microcystin levels in the December tissue samples continued to exceed the Lifetime TDI level for both adults and children.

A review of literature pertaining to microcystin bioaccumulation in freshwater organisms reveals that the microcystin bioaccumulation patterns observed in the Klamath River system are commonly observed in other systems as well, and that World Health Organization guidelines for public health were commonly exceeded in a variety of organisms, including fish and freshwater mussels (Table1). A recent review by Smith et al. (2008) found that in 47% of the aquaculture studies they evaluated, hepatotoxin accumulation occurred in edible tissues that exceeded the WHO TDI (0.04 µg kg⁻¹ body weight d⁻¹; assuming 100-300 g fresh weight of tissue consumed). Further work by Smith et al. (2010) indicates that the majority of microcystins are likely covalently bound to target proteins in tissues and thus are not quantified or included in typical assessments. If, as Smith et al. (2010) indicate, these covalently bound microcystins may be made bioavailable in the digestive system of a consumer through the digestion of their attached protein phosphatase, then public health risk may be underestimated.

SUMMARY

The expanded sampling program in 2009 (relative to 2007) included broader spatial and temporal coverage, and clearly demonstrated bioaccumulation of various microcystin congeners in Klamath River freshwater mussels. Similar to 2007 results, evaluation of bioaccumulation in Klamath River freshwater mussels in 2009 with respect to public health guidelines indicates that, aside from a September sample from the Trinity River, all TDI guideline levels as defined by Ibelings and Chorus (2007) were exceeded to varying degrees, including observations of values exceeding Acute TDI thresholds.

A comparison of tissue concentration data relative to ambient concentration revealed that the congeners MCY-LR and MCY-LA were detected in both media, while MCY-RR and MCY-LRDM were detected in tissue data but they were not detected in water samples. In addition, the frequency of MCY-LR detection in tissue was greater than that for water. The lack of detection or low frequency of microcystin detection in water relative to tissue samples clearly indicates that bioaccumulation mechanisms then cause a proportionally greater frequency of detection (as well as higher concentration) in freshwater mussel tissue. The importance of this finding is that even when microcystin may be below detection in the ambient water, accumulation in freshwater mussel tissue can still occur.

A decline in tissue bioaccumulation was observed in August and coincided with a decline in ambient concentration, as well as a decline in Microcystis aeruginosa cell density and microcystin concentration in the upstream reservoir system during August of 2009. The
Table 1. Literature review of cyanotoxin bioaccumulation in freshwater organisms.

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>System</th>
<th>Toxin</th>
<th>Organism</th>
<th>Exceeds WHO TDI</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorim et al.</td>
<td>1999</td>
<td>fresh water</td>
<td>MC-LR</td>
<td>mussels</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Cazenave et al.</td>
<td>2005</td>
<td>reservoir</td>
<td>MC-RR</td>
<td>fish</td>
<td>yes</td>
<td>Presence of MC-RR in brain</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>2005</td>
<td>lake</td>
<td>MC-LR, MC-RR, MC-YR</td>
<td>mussels</td>
<td>yes</td>
<td>Mean daily intakes were 8-23.5 times TDI value when mussels are eaten whole</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>2006</td>
<td>lake</td>
<td>MC</td>
<td>silver carp</td>
<td>yes</td>
<td>Silver Carp should not be consumed during period of dense Microcystis blooms</td>
</tr>
<tr>
<td>Garcia et al.</td>
<td>2010</td>
<td>lake</td>
<td>MC</td>
<td>blue crab</td>
<td>yes</td>
<td>MC levels exceeded WHO drinking water guidelines.</td>
</tr>
<tr>
<td>Magalhaes et al.</td>
<td>2005</td>
<td>pond</td>
<td>MC-LR, MC-RR, MC-YR</td>
<td>fish</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Magalhaes et al.</td>
<td>2001</td>
<td>coastal lagoon</td>
<td>MC</td>
<td>fish</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Magalhaes et al.</td>
<td>2003</td>
<td>bay</td>
<td>MC</td>
<td>fish, crustaceans</td>
<td>yes</td>
<td>19% of animal samples were above WHO TDI</td>
</tr>
<tr>
<td>Masango et al.</td>
<td>2008</td>
<td>fresh water</td>
<td>MC-LR</td>
<td>fish</td>
<td>yes</td>
<td>MC-LR concentration was 1000 times more than the WHO provisional guideline for drinking water</td>
</tr>
<tr>
<td>Mohamed et al.</td>
<td>2003</td>
<td>fish farm</td>
<td>MC</td>
<td>fish</td>
<td>NA</td>
<td>Highest MC concentration found in guts</td>
</tr>
<tr>
<td>Osswald et al.</td>
<td>2008</td>
<td>laboratory aquarium</td>
<td>Anatoxin-a</td>
<td>mussels</td>
<td>no</td>
<td>One day after beginning depuration the toxin could not be detected</td>
</tr>
<tr>
<td>Valeria et al.</td>
<td>2010</td>
<td>lake</td>
<td>MC-LR, MC-RR, MC-YR, MC-LA</td>
<td>fish</td>
<td>no</td>
<td>MC-RR was dominant in water samples.</td>
</tr>
<tr>
<td>Vasconcelos et al.</td>
<td>1999</td>
<td>estuary</td>
<td>MC-LR</td>
<td>mussels</td>
<td>yes</td>
<td>96% of toxin found in digestive gland and stomach</td>
</tr>
<tr>
<td>Vasconcelos</td>
<td>1999</td>
<td>fresh water</td>
<td>MC-LR</td>
<td>fish, crayfish, mussels</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Wilson et al.</td>
<td>2008</td>
<td>lake</td>
<td>MC</td>
<td>fish</td>
<td>no</td>
<td>MC levels exceeded WHO drinking water guidelines.</td>
</tr>
<tr>
<td>Wood et al.</td>
<td>2006</td>
<td>lake</td>
<td>MC</td>
<td>fish, mussels</td>
<td>NA</td>
<td>MC detected in mussels but not in fish</td>
</tr>
<tr>
<td>Xie et al.</td>
<td>2005</td>
<td>lake</td>
<td>MC-LR, MC-RR</td>
<td>fish</td>
<td>yes</td>
<td>MC found in bile and blood</td>
</tr>
<tr>
<td>Zhao et al.</td>
<td>2006</td>
<td>flow-through</td>
<td>MC</td>
<td>fish</td>
<td>yes</td>
<td></td>
</tr>
</tbody>
</table>
monthly pattern in total microcystin concentration in freshwater mussel tissue indicated relatively high values in July, September, and October, with highest overall values occurring in September and October of 2009. Microcystin was still detected in mussels from the December sample, with several values continuing to exceed the Lifetime TDI for a child.

An overall comparison of ambient data to the tissue data revealed that despite relatively low ambient concentrations (median microcystin values were often less than 1 µg/L), substantial microcystin bioaccumulation occurred at all stations. This was especially true for the most downstream station, KA, where numerous non-detects and low microcystin values were observed (stations KA and TG), yet levels found in freshwater mussel tissue were substantially above the various public health thresholds for safe consumption.

These data show that ingestion of freshwater mussels in the Klamath River system would result in microcystin doses that exceed various public health thresholds for safe consumption throughout the summer and fall. This is true especially for children in the months of July, September, and October, when the Acute Tolerable Intake (TI) dose was exceeded by up to ~4 times. These are the months when traditional and subsistence use of fresh water mussels by Tribal members occurs, and at these times even one meal could exceed safe consumption levels. It should be realized that if the use of these organisms is curtailed during these months that coincide with harvest times, their use would be effectively eliminated both from a dietary and Tribal cultural standpoint. Regardless, given that ambient microcystin levels below public health guidelines for recreation can results in substantial microcystin bioaccumulation freshwater mussels, caution must be exercised when consuming these organisms in the mainstem Klamath River system.

Acknowledgements

We want to thank Grant Johnson, Luana Hillman, Tessa Donahue, and Alex Corum of the Karuk Tribe Field Crew; and Scott Sinnott and Matt Hannington of the Yurok Tribe Field Crew for collection of freshwater mussels and ambient microcystin data. Lisa Bowater provided valuable graphical and word processing assistance to Aquatic Ecosystem Sciences LLC. Funding was provided through the Klamath Hydroelectric Settlement Agreement in Principle (AIP), Interim Measure 12, as presented in the 2009 AIP Monitoring Plan.
LITERATURE CITED


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In response to a request by the North Coast Regional Water Board (Water Board), the Office of Environmental Health Hazard Assessment (OEHHA) is providing general guidance on the application of the provisional tolerable daily intake (TDI) of microcystin-LR (MC-LR) that has been recommended by the World Health Organization (WHO). This guidance is based on WHO (1999) and is intended to assist the Water Board in addressing the 2008 bloom of cyanobacteria on the Klamath River. The provisional TDI provided by WHO (1999) is currently the best available guideline; no other major public health organization has published a TDI or comparable guideline for microcystin exposure at this time. The information presented here represents a general interpretation of WHO recommendations, but does not represent an official OEHHA recommendation.

The WHO has published a provisional TDI for MC-LR of 0.04 \( \mu g/kg/day \) (WHO, 1999). The provisional TDI was derived following standard risk assessment protocol as described below.

1. The most appropriate study representing the relationship between dose and toxicological response was found to be a 13-week MC-LR oral exposure in mice (Fawell et al., 1994 as described in Fawell et al., 1999).

2. The No Observed Adverse Effect Level (NOAEL) was identified. The NOAEL is determined as the highest dose of a chemical that does not induce the targeted toxic effect. In the Fawell et al. (1994) study, 40 \( \mu g \) MC-LR per kg of mouse body weight was the highest dose of MC-LR that did not cause liver injury.

3. An uncertainty factor (UF) of 1000 was applied to the NOAEL, as is typical when applying limited animal data to this type of human risk assessment. This UF incorporates three major areas of uncertainty: 1) humans could be more sensitive to MC-LR toxicity than mice, 2) some humans are potentially more sensitive to MC-LR than others (i.e., protection of the most sensitive people) and 3) additional studies could reveal adverse
effects at lower dosages. The NOAEL was reduced by a factor of 1000 to safeguard against the three uncertainties described above, resulting in a TDI of 0.04 µg/kg-day.

The WHO TDI can be used to calculate the maximum recommended consumption rate of fish or shellfish from an affected water body. For a seasonal bloom such as that occurring on the Klamath, this represents a conservative approach since the TDI is based on a longer exposure period than is expected during the bloom season. The study used by WHO (1999) to derive the TDI (Fawell et al., 1994) is categorized as a chronic study because the exposure lasted more than ten percent of the organism’s average lifespan (13 weeks corresponds to roughly 13 percent of the average life span in mice). Seasonal exposures to microcystin in humans are more likely to be categorized as subchronic (exposures between 1 month and ten percent of lifespan) or short-term (exposures up to 30 days). Another issue is the application of a TDI derived for one microcystin variant (MC-LR) to all of the microcystin variants present in the bloom. Although many variants have been shown to cause acute toxicity similar to MC-LR, WHO (1999) determined that the existing data on other variants was insufficient to derive TDI values. The TDI was used to derive a consumption rate as follows:

\[
CR (\text{kg/day}) = \frac{\text{TDI} (\mu g/\text{kg-day}) \times \text{BW (kg)}}{\text{MC}_{\text{edible}} (\mu g/kg)}
\]

where,
- \( \text{CR} \) = consumption rate,
- \( \text{TDI} \) = tolerable daily intake,
- \( \text{BW} \) = body weight,
- \( \text{MC}_{\text{edible}} \) = concentration of MC in edible portions of fish or shellfish (fresh weight).

To calculate \( \text{CR} \) as oz/month use:

\[
\text{CR (oz/mo.)} = \frac{\text{TDI} (\mu g/\text{kg-day}) \times \text{BW (kg)} \times 1,073 \text{ oz/mo}^+}{\text{MC}_{\text{edible}} (\mu g/kg)}
\]

\(^+\) Calculated as follows: 1 kg/day * 30.4 days/mo * 35.3 oz/kg = 1073 oz/mo.
And finally, to calculate CR as the number of 8-oz meals/month use:

\[
CR (\text{# 8-oz meals/mo.}) = \frac{TDI (\mu g/\text{kg-day}) \times BW (\text{kg}) \times 134 \text{ meals/mo}}{MC_{\text{edible}} (\mu g/\text{kg})}
\]

* Calculated as follows: 1 kg/day * 30.4 days/mo * 35.3 oz/kg * 1 meal/8oz = 134 8-oz meals/mo.

The above equation was used to calculate the maximum number of 8-oz meals per month from affected areas, with the results shown in the following table (exact values shown in parentheses):

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<th>Adult ‡</th>
<th>Child §</th>
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<td>10</td>
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<tr>
<td>20</td>
<td>19 (19)</td>
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<tr>
<td>40</td>
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<td>60</td>
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<tr>
<td>500</td>
<td>&lt; 1 (0.8)</td>
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</table>

‡ Adult = 70 kg (~155 lbs)  § Child = 10 kg (~ 22 lbs)

References

Fawell, J.K., James, C.P. and James, H.A. 1994 Toxins from Blue-Green Algae: Toxicological Assessment of Microcystin-LR and a Method for its Determination in Water, Water Research Centre, Medmenham, UK.


cc: James C. Carlisle, D.V.M., Chief
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