

**Date:** June 10, 2011

**From:** Wayne W. Carmichael  
Professor Emeritus

**Subject:** Peer Review of Cyanotoxin Toxicity Criteria and Health Based Water Concentrations to Protect Human Swimmers, Dogs and Cattle.

**Prepared for:** State Water Resources Control Board-Division of Water Quality

**Att:** Dominic Gregorio  
Senior Environmental Scientist  
Chief, Ocean Unit

**Summary:** In organizing and presenting comments to the cyanotoxin toxicity criteria document I have focused on the major points requested-namely toxicity criteria, exposure criteria and the general approach used by OEHHA in writing the document. To accomplish these goals I have added detail where needed, additional text or edited text, plus references to support the new or edited text. I believe my edits will contribute to a more accurate, usable and defensible document for setting reference doses (RfD) and the use of those RfDs to estimate maximum concentration levels. These in turn will help protect the public's health from Cyanobacteria Harmful Algae Blooms (CyanoHABs) and their toxins.

**Review Contents:**

**Comments on the General Approach Used for Action Level Development:** OEHHA has limited the scope of the cyanotoxin assessment to four forms of microcystins plus anatoxin-a and cylindrospermopsin. There at least 76 forms of microcystins (Mcysts) but the four under consideration (Mcyst-LA, YR, RR and LR) are the most commonly found ones in U.S. and California waters. Comments for cylindrospermopsin are generic even though there are two known variants, 7-epicylindrospermopsin, with equal toxicity to cylindrospermopsin, and deoxycylindrospermopsin with lower toxicity than other two (Meriluoto and Codd, 2005). Likewise for anatoxin-a, criteria was developed based on only anatoxin-a. In addition to anatoxin-a, Homoanatoxin-a and 4-hydroxyhomoanatoxin-a have been described. Some photodegradation products of anatoxin-a, namely dihydroanatoxin and epoxyanatoxin have also been identified (Meriluoto and Codd, 2005).

Two of these three cyanotoxin groups are currently the most common found in U.S. and California water supplies-the microcystins and anatoxin-a. Of the microcystins, the four reviewed are also the most common found. These two groups plus cylindrospermopsin are the top three priorities for health risk and for detection methods development as listed by the U.S. EPA. It is therefore appropriate and prudent that these toxin groups be the ones reviewed by OEHHA. However some cautions involving occurrence, health

risk and legal points should be considered as the document is developed and considered for adoption. These include:

- 1) Cylindrospermopsin was placed on the EPA priority list because it was perceived to be an emerging cyanotoxin with regards to occurrence and hazard. To date it has not been identified in California waters and in only a few U.S. water supplies (i.e. Florida, Indiana and Oregon).
- 2) If these become the only cyanotoxins monitored for, it is very likely some will be missed (i.e. other microcystins, anatoxin-a(s) and saxitoxins), in any monitoring program based upon assessment of only these 6 cyanotoxins, and possible guidelines or regulations that may be adopted based upon an evaluation of these 6 cyanotoxins.
- 3) Because only 6 cyanotoxins are being reviewed and assessed there may be legal issues that arise from occurrences, exposures and/or toxicities due to Cyanobacteria Harmful Algae Blooms (CyanoHABs) that contain other cyanotoxins.

### **Comments on Toxicity Criteria Used for the Six Chemicals.**

The reasoning and text for the Toxicity Criteria-Assessment (pages 10-21) is overall very good and complete. Acute and acute-lethal poisoning from microcystins are the only toxicities that have been confirmed. Liver carcinogenesis has not been demonstrated except in laboratory experiments and then only when initiation from a proven carcinogen such as aflatoxin is also used. There is however one statement that does need editing. On page 11 under “Existing Health-Based Criteria” – the sentence **“WHO (2) considered the ability of microcystins to promote liver tumors, but the international Agency for Research on Cancer found the evidence for microcystins to cause cancer in humans inadequate”**-is correct in that WHO did discuss the topic but did not consider it. and it is correct that IARC found the evidence inadequate— however the two are not linked in the sense that one might have influenced the other. WHO did their study in 1998 and published it in 1999 (ref 2). IARC did their evaluation in 2005 and published it in 2006 (ref 62). The real reason WHO did not act on any evidence for linking microcystins to cancer was that the Australian representatives to the WHO deliberations were explicitly asked not to consider the question of microcystins and cancer. It is therefore more correct to say that WHO simply did not address the issue at all, following the Australian request against it.

The use of cyanobacterial extracts (generally greater than 90% pure) is an acceptable criteria for assessing cyanotoxin action levels. There are no certified reference standards for any cyanotoxins. The best reference materials are 95% pure (or better)- however they have not been quality controlled (certified) by more than two methods- usually HPLC peak purity or by the use of extinction coefficients. In turn purity of these standards have been determined using reference materials not certified by NMR or LC/MS.

### **Comments on Exposure Assessment Assumptions**

Addressing exposure assessment for humans (recreational waters), and domestic animals (livestock and pets), covering pages 21-29, is a good approach. This reviewer finds the calculations for water intake and related action levels determined to be the best ones possible given the available data on toxicity and exposure scenarios. Likewise the Exposure calculations in Appendix I through VI (pages 30-46) are also appropriate. The professional judgements used in estimating exposure to dogs is acceptable to this reviewer. The only caveat to this, from this reviewer, is that dogs do exhibit a fairly rapid acute toxicity from licking fur matted with bloom material that contains anatoxin-a or anatoxin-a(s). Dogs are also attracted to fermenting mats of cyanobacteria near shorelines of waterbodies. In other words-dogs may be unusually sensitive to cyanotoxin neurotoxins. This attraction and rapid toxicity was discussed in a paper by Codd et al. 1992:

Codd, G.A. , Edwards, C., Beattie, K.A. Barr, W.M., and Gunn, GJ. (1992) Fatal Attraction to Cyanobacteria? Nature. 359:110-111.

### **Comments on Microcystins, Anatoxin-a and Cylindrospermopsin Ecotoxicology**

An assessment of the ecotoxicology of cyanotoxins is a very important topic. Indeed it may be even more important than the risk to humans and domestic animals. This is primarily because human activities leading to eutrophication and alteration of water supplies are the primary drivers for the increased incidence and duration of Cyanobacteria Harmful Algae Blooms (CyanoHABs). Aquatic and terrestrial systems are widely affected by CyanoHABs. However as the review points out on page 46-the topic is “complex and evolving”. Equally important to setting action levels to reduce adverse health effects of cyanotoxins should be programs and actions to reduce human impacts on aquatic systems that are responsible for the increases in CyanoHABs and their significant impacts on natural populations of plants and animals. While there are many more examples and studies that show ecotoxicological effects from cyanotoxins the discussion and examples on pages 51-72 are good examples to have used for the document.

**Specific Editing Changes to the Document:** Some general edits are needed in the document. These are as follow:

- 1) Page 2-line line 8. The number of papers reviewed by OEHHA (2025) represents about half of the scientific papers on the topic to 2004. It is estimated, that about another 2000 have been published since 2004, meaning the OEHHA review is based on about one-third of the available publications. The initial 2004 publication list is available for the USEPA at;  
[http://nlquery.epa.gov/epasearch/epasearch?typeofsearch=epa&filterclause=%28tssms:ogwdw000%29%20AND%20&max\\_results=100&referer=http%253A%252F%252Fwww.epa.gov%252Fsafewater%252Findex.html&result\\_template=epafiles\\_default.xsl&areaname=Ground%20Water%20%20%20Drinking%20Water&areapagehead=epafiles\\_pagehead&areapagefoot=epafiles\\_pagefoot&areasidebar](http://nlquery.epa.gov/epasearch/epasearch?typeofsearch=epa&filterclause=%28tssms:ogwdw000%29%20AND%20&max_results=100&referer=http%253A%252F%252Fwww.epa.gov%252Fsafewater%252Findex.html&result_template=epafiles_default.xsl&areaname=Ground%20Water%20%20%20Drinking%20Water&areapagehead=epafiles_pagehead&areapagefoot=epafiles_pagefoot&areasidebar)

[=search\\_sidebar&stylesheet=http://www.epa.gov/epafiles/s/epa.css&sort=term\\_relevancy&faq=no&results\\_per\\_page=10&cluster=both&sessionid=FB8AEAD369DCEA87FAF295DEC7CBA1CB&querytext=cyanobacteria%20toxins&start=11&doctype=all](http://www.epa.gov/epafiles/s/epa.css&sort=term_relevancy&faq=no&results_per_page=10&cluster=both&sessionid=FB8AEAD369DCEA87FAF295DEC7CBA1CB&querytext=cyanobacteria%20toxins&start=11&doctype=all)

- 2) Page 4. Table 1. The molecular weights reported for the four microcystins vary with instrument at the decimal point numbers. It is best to omit the mass fraction numbers (i.e. 910 not 910.06). Also the Molecular Weight reported is actually mass plus 1. Therefore the column heading should be changed to read: "Molecular Weight plus H".
- 3) Page 5. Line 4. It is now accepted that the species *Aphanizomenon flos-aquae* is not a known toxin producer. Previous references to it as a producer have proven to be other species in the genus or from mixed populations where another cyanobacteria actually was the toxin producer. For example see ref:

Carmichael, W.W. 2001. Health Effects of Toxin Producing Cyanobacteria: "The CyanoHABS". Human and Ecological Risk Assessment. 7(5): 1393-1407.

Li, R., Carmichael, W.W., Liu, Y. and Watanabe, M.M. 2000. Taxonomic re-evaluation of *Aphanizomenon flos-aquae* NH-5 based upon morphology and 16S rRNA gene sequences. Hydrobiologia, 438(1): 99-105.

Li, R.H. and W.W. Carmichael. 2003. Morphological and 16S rRNA gene evidence for reclassification of the paralytic shellfish toxin producing *Aphanizomenon flos-aquae* LMECYA 31 as *Aphanizomenon issatschenkoj*. J. of Phycol. 39. 814-818.

- 4) Page 6. Line 14. There is some published material on biodegradation of cylindrospermopsin. One paper by Woermer et al found no bacterial degradation over 40 days. See below references:

Smith, M. J., Shaw, G. R., Eaglesham, G. K., Ho, L., and Brookes, J. D. (2008). Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. Environ. Toxicol. 23, 413-421.

Wormer, L., Cires, S., Carrasco, D., and Quesada, A. (2008). Cylindrospermopsin is not degraded by co-occurring natural bacterial communities during a 40-day study. Harmful Algae 7, 206-213.

- 5) Page 7. Line 8. It is my understanding that Clear Lake in Northern California has a history of microcystin occurrence. Also a just published paper details microcystin in Pinto lake and transfer to Monterey Bay –see:

Miller MA, Kudela RM, Mekebri A, Crane D, Oates SC, et al. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land

to Sea Otters. (2010) PLoS ONE 5(9): e12576.  
doi:10.1371/journal.pone.0012576

- 6) Page 7. Bottom paragraph. A more complete ref for the Brazil human deaths is Carmichael et al. 2001. In this outbreak report 100 patients developed acute liver failure (of 116/131 with symptoms) – 76 died and 52 were confirmed with cyanotoxin poisoning. See:

Carmichael, W.W., Azevedo, M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Egelsham, G.K. 2001 Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins. *Environmental Health Perspectives*. 109 (7):663-668.

- 7) page 9-dog deaths. Oregon has also reported dog deaths from anatoxin-a. see: <http://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Pages/new2009.aspx>
- 8) page 68-Food Web Transfer. The recent exposure of microcystins to Sea Otters in Monterey Bay should be included in this section. See: Miller MA, Kudela RM, Mekebri A, Crane D, Oates SC, et al. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. (2010) PLoS ONE 5(9): e12576. doi:10.1371/journal.pone.0012576
- 9) Terminology-page 48-51. On page 48 the definition of purified toxin should be modified to indicate an important topic in developing methods of analysis and toxicology mechanisms. There is a distinction between “reference standards” and “certified reference material”. Purified toxin does not indicate degree of purity and this varies widely from different sources using different extraction methods and whether multiple methods for quality control have been used. Reference standards do not carry the same degree of purity testing and usual only have had one or two QC methods applied-i.e. HPLC purity as compared against another reference material whose purity might be no more than 90-95%. Certified Reference materials would have multiple QC methods applied-i.e. HPLC, extinction coefficient. LC-MS or MS-MS and even NMR. These “standards” would be used as the ultimate comparison for purity of an extract and should be 99% or better pure. For some applications reference materials are suitable but for others only certified reference material should be used. Therefore it is suggested the terms “Reference Standard and “Certified Reference Standard” be put in this table.
- 10)Page 13-Microcystins and Cancer. It is true that the question of cancer has been addressed by IARC and OEHHA has handled the question appropriately. There is a new review published on the topic which might be good to consider and insert in this document. It is:

Z̄ egura, B., S̄ traser, A., Filipic, M.. 2011. Genotoxicity and potential carcinogenicity of cyanobacterial toxins – a review. Mutation Research/Reviews in Mutation Research. 727:1-2. 16-41.

### **References Cited in this Editors Review:**

Meriluoto, J. and Codd, G.A. 2005. Cyanobacterial Monitoring and Cyanotoxin Analysis. Abo Akademi University Press. ISSN 0001-5105; Vol. 65, no. 1. Pp. 148.

Miller MA, Kudela RM, Mekebri A, Crane D, Oates SC, et al. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. (2010) PLoS ONE 5(9): e12576. doi:10.1371/journal.pone.0012576

Carmichael, W.W. 2001. Health Effects of Toxin Producing Cyanobacteria: "TheCynoHABS". Human and Ecological Risk Assessment. 7(5): 1393-1407.

Li, R., Carmichael, W.W., Liu, Y. and Watanabe, M.M. 2000. Taxonomic re-evaluation of *Aphanizomenon flos-aquae* NH-5 based upon morphology and 16S rRNA gene sequences. Hydrobiologia, 438(1): 99-105.

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Carmichael, W.W., Azevedo, M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eagelsham, G.K. 2001 Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins. Environmental Health Perspectives. 109 (7):663-668.

Smith, M. J., Shaw, G. R., Eaglesham, G. K., Ho, L., and Brookes, J. D. (2008). Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. Environ. Toxicol. 23, 413-421.

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Codd, G.A. , Edwards, C., Beattie, K.A. Barr, W.M., and Gunn, GJ. (1992) Fatal Attraction to Cyanobacteria? Nature. 359:110-111.

**Respectfully Submitted**

A handwritten signature in cursive script that reads "Wayne W. Carmichael". The signature is written in a dark ink and has a long, sweeping underline that extends across the width of the name.

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