Children's Health Protection Advisory Committee

Chair:

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Administrator Gina McCarthy
United States Environmental Protection Agency
1200 Pennsylvania Ave, NW
Washington, DC 20460

RE: CHPAC Comments on Cyanotoxin Drinking Water Health Advisories

Dear Administrator McCarthy:

Thank you for the opportunity to respond to your questions regarding the drinking water health advisories for two cyanotoxins, microcystin and cylindrospermopsin, issued by the Office of Water in June 2015.

We are pleased to see both advisories issued. Cyanotoxins are an important emerging public health issue, especially for children. The advisories will assist state and local officials in managing risk from these contaminants in drinking water. We address each of your three charge questions below.

Charge Question #1: In considering the current peer-reviewed literature and the available public documentation, does the drinking water health advisory adequately protect early lifestages at increased exposure and/or toxicity from cyanotoxins? Specifically, has EPA appropriately described the data on early life susceptibility, uncertainty associated with the limited data in the application of the database uncertainty factor, and the use of infant drinking water ingestion rates?

EPA has been a leader in recognizing that children have higher relative exposure than adults to contaminants in water, food, and air. The agency's guidance documents, including its 2011 *Exposure Factors Handbook*,[1] have provided essential estimates of childhood exposure in a variety of situations.

In both of the cyanotoxin health advisories, EPA took a reasonable approach to protecting children by relying on age-specific exposure factors from the Handbook including: drinking water use for formula-fed infants, body weights of younger children, and the increased ratio of drinking water intake per unit body weight of children. We appreciate this effort to incorporate early life stages into Agency advice for the protection of children. We recognize that the Agency concluded that information about drinking water consumption for children from birth to three months of age

[1] EPA, Exposure Factors Handbook: 2011 Edition, EPA/600/R-090/052F, 2011. See http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252#Download

was limited; therefore, the advisories were based on consumption patterns from birth to six months of age. CHPAC recommends that the Agency strengthen the estimate of water intake data for children from birth to three months of age.

Regarding the microcystin health advisory, the lack of data on developmental endpoints is concerning. Results of the reproductive study by Chen et al. (2011) indicate that microcystin may disrupt male hormones and reproductive function. This study has implications for developmental endpoints as androgen disruption during critical periods of fetal development may cause malformations and persistent dysfunction in reproductive tissue. CHPAC recommends that EPA address this important data gap to ensure protection of sensitive developmental windows in both the fetal and early childhood periods.

In addition, CHPAC is concerned about potential carcinogenicity of both cyanotoxins. In the microcystin health advisory, EPA noted that the International Agency for Research on Cancer (IARC) classified it as a Group 2B carcinogen (possibly carcinogenic to human) based on strong evidence supporting a plausible liver tumor promotor mechanism. However, EPA chose not to classify it as a carcinogen without stronger epidemiologic data and a chronic bioassay of purified microcystin. CHPAC has concerns with cylindrospermopsin due to its genotoxicity.

For both microcystin and cylindrospermopsin, CHPAC recommends that EPA reconsider carcinogenicity risks to children posed by genotoxic cyanotoxins and their health advisories. Furthermore, cyanobacterial blooms can produce multiple cyanotoxins, thus CHPAC recommends that EPA consider the potential combined effects, particularly concerning genotoxicity and carcinogenicity.

Moreover, CHPAC recommends that EPA reevaluate the current short-term health advisory framework to more adequately protect against repeated exposures of any genotoxic agents that may have a cumulative effect.

Charge Question #2: Based on the available literature, are there other routes of exposure to children or sensitive child health endpoints EPA should be considering in the advisory for cyanotoxins?

In our response to the previous charge, CHPAC discussed our recommendations that EPA consider additional child-sensitive endpoints such as developmental toxicity, genotoxicity, and carcinogenicity.

For both health advisories, CHPAC conducted a literature review to identify additional relevant research. Studies that CHPAC identified were mostly published after the May 2014 cutoff point for EPA's literature search. The searches resulted in 52 peer-reviewed publications that were not cited and provide potentially relevant mechanistic information that could address some of the data gaps (See Attachment A).

CHPAC understands that drinking water health advisories, including the ones for cylindrospermopsin and microcystin, assume that drinking water is the only route of exposure. In contrast, drinking water maximum contaminant levels (MCL) consider other routes of exposure and, through the relative source contribution (RSC), start with the assumption that drinking water represents only 20% of overall exposure.

CHPAC is concerned that the health advisories ignored combined exposure from drinking water and recreational activities. Each exposure route is a potentially important source of

children's exposure because cyanobacterial blooms are seasonal, and combined exposure is likely to occur. Children are at an increased risk for exposure to cyanotoxins during blooms in recreational water as they may not read or heed posted signs, are more likely to wade into and explore a surface scum, and are more likely to swallow water during swimming and water play.[2] Routes of exposure for children's recreational exposure to water include direct dermal contact, inhalation, and ingestion during recreational activities. CHPAC recommends that EPA include incidental ingestion from children's recreational activities when estimating children's exposure for drinking water health advisories.

Charge Question #3: How can EPA assist States and public water systems to best communicate the children's health risks of cyanotoxins to the public?

CHPAC commends EPA on the outreach already launched to assist states and public water supply managers on cyanotoxins. This includes EPA support of cyanotoxin webpages, newsletters, listserves, workshops, and webinars. EPA should continue to provide assistance to states on outreach and risk communication, especially during outbreaks.

CHPAC recommends that EPA conduct outreach to healthcare providers to improve clinical recognition and management of cyanotoxin illness. A number of EPA documents advise the public to contact their provider promptly if they have symptoms. However, CHPAC found no published guidance to healthcare providers about the questions that would be most useful to include in an exposure history relative to contact with recreational or drinking water; whom to contact if they suspect a case of cyanotoxin illness; whether diagnostic testing for exposure or health effects is available; or how to manage patient symptoms and monitor for recovery. CHPAC recommends that EPA consult with the American Academy of Pediatrics, the Centers for Disease Control and Prevention (CDC), and the Pediatric Environmental Health Specialty Units (PEHSUs) to develop guidance for clinicians. The guidance could be posted on the CDC website and distributed through the PEHSUs and other state and local public health networks.

Finally, in additional to the drinking water health advisories, there is an urgent need for EPA to develop science-based guidance for cyanotoxins in recreational water because of the potential health risk. Currently, state guidelines vary widely (http://www2.epa.gov/nutrient-policy-data/guidelines-and-recommendations). A uniform EPA standard based on the best available science will clarify advice for the public and provide guidance for people in the 30 states that have yet to issue guidance. Therefore, CHPAC recommends that EPA develop guidance for exposure to cyanotoxins from recreational use.

Overall, the drinking water health advisories represent an important step forward in their use of child-specific exposure factors and a good example of effective communications on a complicated issue. To summarize, CHPAC recommends that EPA:

- Do what is necessary to strengthen the estimate of water intake for children from birth to age three months;
- Address data gaps to ensure protection of sensitive developmental windows in both the fetal and early childhood periods;
- Reconsider carcinogenicity risks to children posed by even short-term exposures to the cyanotoxins;
- Consider the potential combined effects of multiple cyanotoxins, particularly concerning genotoxicity and carcinogenicity;

[2] U.S. EPA. Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F, 2011.

- Reevaluate the current short-term health advisory framework to more adequately protect against short-term, repeated exposures of genotoxic agents that may have a cumulative effect;
- Include incidental ingestion from children's recreational activities when estimating children's exposure for drinking water health advisories;
- Continue to provide assistance to states on outreach and risk communication, especially during outbreaks;
- Conduct outreach to health care providers to improve clinical recognition and management of cyanotoxin illness;
- Work with the American Academy of Pediatrics, the Centers for Disease Control and Prevention (CDC), and the Pediatric Environmental Health Specialty Units (PEHSUs) to develop guidance for clinicians; and
- Develop guidance for exposure to cyanotoxins from recreational use.

CHPAC appreciates this opportunity to comment on the documents and we thank you for your commitment to children's health.

Sincerely,

Sheela Sathyanarayana, M.D., M.P.H.
Chair

cc: Betsy Behl, Director, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division
Eric Burneson, Director, Office of Water, Office of Ground Water and Drinking Water, Standards and Risk Management Division
Lesley D'Anglada, Microbiologist, Office of Water, Office of Science and Technology
Ruth Etzel, Director, Office of Children's Health Protection
Peter Grevatt, Director, Office of Water, Office of Ground Water and Drinking Water
Jacqueline Moya, Environmental Engineer, Office of Research and Development, National Center for Environmental Assessment
Elizabeth Southerland, Director, Office of Water, Office of Science and Technology
Jamie Strong, Branch Chief, Office of Water, Office of Science and Technology

Attachment A
Results of CHPAC Literature Review for Microcystin and Cylindrospermopsin

In July 2015, CHPAC conducted a literature search and compared the results to the citations in the microcystin and cylindrospermopsin drinking water health advisories. The search resulted in 52 peer-reviewed publications that were not cited and provide potentially relevant mechanistic information that could address some of the data gaps.

Microcystin - Results of Literature Search		
Title	**Author**	**Citation**
Mechanisms of microcystin-LR-induced cytoskeletal disruption in animal cells.	Zhou et al.	Toxicon. 2015 Jul;101:92-100. doi: 10.1016/j.toxicon.2015.05.005. Epub 2015 May 14. Review. PMID: 25981867
Involvement of oxidative stress and cytoskeletal disruption in microcystin-induced apoptosis in CIK cells.	Chen et al.	Aquat Toxicol. 2015 May 14;165:41-50. doi: 10.1016/j.aquatox.2015.05.009. [Epub ahead of print] PMID: 26022555
The role of GSH in microcystin-induced apoptosis in rat liver: Involvement of oxidative stress and NF-κB	Chen et al.	J. Environ Toxicol. 2014 Nov 20. doi: 10.1002/tox.22068. [Epub ahead of print] PMID: 25410294
MCLR-induced PP2A inhibition and subsequent Rac1 inactivation and hyperphosphorylation of cytoskeleton-associated proteins are involved in cytoskeleton rearrangement in SMMC-7721 human liver cancer cell line	Wang et al.	Chemosphere. 2014 Oct;112:141-53. doi: 10.1016/j.chemosphere.2014.03.130. Epub 2014 May 8. PMID: 25048900
NF-κB plays a key role in microcystin-RR-induced HeLa cell proliferation and apoptosis.	Chen et al.	Toxicon. 2014 Sep;87:120-30. doi: 10.1016/j.toxicon.2014.06.002. Epub 2014 Jun 14. PMID: 24932741
Endocrine-disrupting effects and reproductive toxicity of low dose MCLR on male frogs (Rana nigromaculata) in vivo	Jia et al.	Aquat Toxicol. 2014 Oct;155:24-31. doi: 10.1016/j.aquatox.2014.06.002. Epub 2014 Jun 13. PMID: 24971790
Regulation of microcystin-LR-induced toxicity in mouse spermatogonia by miR-96.	Zhou et al.	Environ Sci Technol. 2014 Jun 3;48(11):6383-90. doi: 10.1021/es500152m. Epub 2014 May 13. PMID: 24803159
Survival, growth and toxicity of Microcystis aeruginosa PCC 7806 in experimental conditions mimicking some features of the human gastro-intestinal environment	Stefanelli et al.	Chem Biol Interact. 2014 May 25;215:54-61. doi: 10.1016/j.cbi.2014.03.006. Epub 2014 Mar 22. PMID: 24667652
Genotoxicity of microcystin-LR in in vitro and in vivo experimental models	Dias et al.	Biomed Res Int. 2014;2014:949521. doi: 10.1155/2014/949521. Epub 2014 May 18. PMID: 24955368
Overexpression of Nrf2 protects against microcystin-induced hepatotoxicity in mice.	Lu et al.	PLoS One. 2014 Mar 25;9(3):e93013. doi: 10.1371/journal.pone.0093013. eCollection 2014. PMID: 24667526
Selectivity and potency of microcystin congeners against OATP1B1 and OATP1B3 expressing cancer cells	Niedermeyer	PLoS One. 2014 Mar 10;9(3):e91476. doi: 10.1371/journal.pone.0091476. eCollection 2014. PMID: 24614281
Microcystin-LR stabilizes c-myc protein by inhibiting protein phosphatase 2A in HEK293 cells	Fan et al.	Toxicology. 2014 May 7;319:69-74. doi: 10.1016/j.tox.2014.02.015. Epub 2014 Mar 7. PMID: 24607848
Dualistic evolution of liver damage in mice triggered by a single sublethal exposure to Microcystin-LR	Mattos et al.	Toxicon. 2014 Jun;83:43-51. doi: 10.1016/j.toxicon.2014.02.015. Epub 2014 Mar 1. PMID: 24593963

Microcystin-LR affects properties of human epidermal skin cells crucial for regenerative processes	Kozdeba et al.	Toxicon. 2014 Mar;80:38-46. doi: 10.1016/j.toxicon.2014.01.003. Epub 2014 Jan 21. PMID: 24462717
Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in Daphnia magna and the role of microcystins.	Smutna et al.	Toxicon. 2014 Mar;79:11-8. doi: 10.1016/j.toxicon.2013.12.009. Epub 2014 Jan 8. PMID: 24412459
Effects of the amino acid constituents of microcystin variants on cytotoxicity to primary cultured rat hepatocytes	Shimizu et al.	Toxins (Basel). 2013 Dec 30;6(1):168-79. doi: 10.3390/toxins6010168. PMID: 24380975
Assessment of the mutagenic potential of cyanobacterial extracts and pure cyanotoxins	Sieroslawska	Toxicon. 2013 Nov;74:76-82. doi: 10.1016/j.toxicon.2013.07.029. Epub 2013 Aug 7. PMID: 23933197
The conjugation of microcystin-RR by human recombinant GSTs and hepatic cytosol.	Buratti et al.	Toxicol Lett. 2013 Jun 7;219(3):231-8. doi: 10.1016/j.toxlet.2013.03.015. Epub 2013 Mar 25. PMID: 23538035
Mitochondrial and endoplasmic reticulum pathways involved in microcystin-LR-induced apoptosis of the testes of male frog (Rana nigromaculata) in vivo	Zhang et al.	J Hazard Mater. 2013 May 15;252-253:382-9. doi: 10.1016/j.jhazmat.2013.03.017. Epub 2013 Mar 16. PMID: 23548922
Dog poisonings associated with a Microcystis aeruginosa bloom in the Netherlands	Lürling et al.	Toxins (Basel). 2013 Mar 14;5(3):556-67. doi: 10.3390/toxins5030556. PMID: 23493170
Comparative cellular toxicity of hydrophilic and hydrophobic microcystins on Caco-2 cells.	Vesterkvist et al.	Toxins (Basel). 2012 Oct 25;4(11):1008-23. doi: 10.3390/toxins4111008. PMID: 23202304
Microcystin-LR induced DNA damage in human peripheral blood lymphocytes	Zegura et al.	Mutat Res. 2011 Dec 24;726(2):116-22. doi: 10.1016/j.mrgentox.2011.10.002. Epub 2011 Oct 7. PMID: 22001196
The apoptosis-inducing activity towards leukemia and lymphoma cells in a cyanobacterial culture collection is not associated with mouse bioassay toxicity	Oftedal et al.	J Ind Microbiol Biotechnol. 2011 Apr;38(4):489-501. doi: 10.1007/s10295-010-0791-9. Epub 2010 Aug 6. PMID: 20689978
Microcystin-LR induces toxic effects in differentiated and undifferentiated Caco-2 cells.	Puerto et al.	Arch Toxicol. 2010 May;84(5):405-10. doi: 10.1007/s00204-010-0513-0. Epub 2010 Jan 30. PMID: 20112101
Molecular aspects of microcystin-induced hepatotoxicity and hepatocarcinogenesis.	Svircev et al.	J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2010 Jan;28(1):39-59. doi: 10.1080/10590500903585382. Review. PMID: 20390967

Cylindrospermopsin - Results of Literature Search		
Title	**Author**	**Citation**
The role of the enzymatic antioxidant system in cylindrospermopsin-induced toxicity in human lymphocytes.	Poniedziale k et al.	J. Toxicol In Vitro. 2015 Aug;29(5):926-32. doi: 10.1016/j.tiv.2015.03.023. Epub 2015 Apr 9. PMID: 25863213
Cylindrospermopsin induces neurotoxicity in tilapia fish (Oreochromis niloticus) exposed to Aphanizomenon ovalisporum.	Guzmán-Guillén et al.	Aquat Toxicol. 2015 Apr;161:17-24. doi: 10.1016/j.aquatox.2015.01.024. Epub 2015 Jan 31. PMID: 25661706
Low concentrations of cylindrospermopsin induce increases of reactive oxygen species levels, metabolism and proliferation in human hepatoma cells (HepG2).	Liebel et al.	Toxicol In Vitro. 2015 Apr;29(3):479-88. doi: 10.1016/j.tiv.2014.12.022. Epub 2015 Jan 6. PMID: 25575781
Investigating the therapeutic effects of LASSBio-596 in an in vivo model of cylindrospermopsin- induced lung injury.	Oliveira et al.	Toxicon. 2015 Feb;94:29-35. doi: 10.1016/j.toxicon.2014.12.004. Epub 2014 Dec 18. PMID: 25528385
Toxic potencies of metabolite(s) of non-cylindrospermopsin producing Cylindrospermopsis raciborskii isolated from temperate zone in human white cells.	Poniedziałe k et al.	J. Chemosphere. 2015 Feb;120:608-14. doi: 10.1016/j.chemosphere.2014.09.067. Epub 2014 Oct 28. PMID: 25462304
Cylindrospermopsin induces oxidative stress and genotoxic effects in the fish CLC cell line	Sieroslawsk a et al.	J Appl Toxicol. 2015 Apr;35(4):426-33. doi: 10.1002/jat.3040. Epub 2014 Sep 12. PMID: 25219470
The course of toxicity in the pregnant mouse after exposure to the cyanobacterial toxin cylindrospermopsin: clinical effects, serum chemistries, hematology, and histopathology.	Chernoff et al.	J Toxicol Environ Health A. 2014;77(17):1040-60. doi: 10.1080/15287394.2014.919838. PMID: 25072824
Modulation of chromatin remodelling induced by the freshwater cyanotoxin cylindrospermopsin in human intestinal caco-2 cells.	Huguet et al.	PLoS One. 2014 Jun 12;9(6):e99121. doi: 10.1371/journal.pone.0099121. eCollection 2014. PMID: 24921660
Double strand breaks and cell-cycle arrest induced by the cyanobacterial toxin cylindrospermopsin in HepG2 cells	Alja et al.	Mar Drugs. 2013 Aug 21;11(8):3077-90. doi: 10.3390/md11083077. PMID: 23966038
Cylindrospermopsin induced transcriptional responses in human hepatoma HepG2 cells	Straser et al.	Toxicol In Vitro. 2013 Sep;27(6):1809-19. doi: 10.1016/j.tiv.2013.05.012. Epub 2013 May 29. PMID: 23726867
The protective role of l-carnitine against cylindrospermopsin-induced oxidative stress in tilapia (Oreochromis niloticus).	Guzmán-Guillén et al.	Aquat Toxicol. 2013 May 15;132-133:141-50. doi: 10.1016/j.aquatox.2013.02.011. Epub 2013 Feb 27. PMID: 23501490
The influence of cylindrospermopsin on oxidative DNA damage and apoptosis induction in HepG2 cells	Straser et al.	Chemosphere. 2013 Jun;92(1):24-30. doi: 10.1016/j.chemosphere.2013.03.023. Epub 2013 Apr 16. PMID: 23601126
Protein synthesis inhibition and oxidative stress induced by cylindrospermopsin elicit apoptosis in primary rat hepatocytes	López-Alonso et al.	Chem Res Toxicol. 2013 Feb 18;26(2):203-12. doi: 10.1021/tx3003438. Epub 2013 Jan 15. PMID: 23270326
Alterations observed in the endothelial HUVEC cell line exposed to pure Cylindrospermopsin	Gutiérrez-Praena et al.	Chemosphere. 2012 Nov;89(9):1151-60. doi: 10.1016/j.chemosphere.2012.06.023. Epub 2012 Jul 19. PMID: 22818884
Time-dependence of lung injury in mice acutely exposed to cylindrospermopsin	Oliveira et al.	Toxicon. 2012 Oct;60(5):764-72. doi: 10.1016/j.toxicon.2012.06.009. Epub 2012 Jun 26. PMID: 22750219
Cylindrospermopsin, a blue-green algal toxin, inhibited human luteinised granulosa cell protein synthesis in vitro.	Young et al.	Toxicol In Vitro. 2012 Aug;26(5):656-62. doi: 10.1016/j.tiv.2012.03.001. Epub 2012 Mar 10. PMID: 22429990

Biochemical and pathological toxic effects induced by the cyanotoxin Cylindrospermopsin on the human cell line Caco-2	Gutiérrez-Praena et al.	Water Res. 2012 Apr 1;46(5):1566-75. doi: 10.1016/j.watres.2011.12.044. Epub 2011 Dec 30. PMID: 22227240
Genotoxicity and potential carcinogenicity of cyanobacterial toxins - a review	Zegura et al.	Mutat Res. 2011 Jan-Apr;727(1-2):16-41. doi: 10.1016/j.mrrev.2011.01.002. Epub 2011 Jan 26. Review. Erratum in: Mutat Res. 2012 Jan-Mar;750(1):83. PMID: 21277993
Modulation of gap-junctional intercellular communication by a series of cyanobacterial samples from nature and laboratory cultures	Nováková et al.	Toxicon. 2011 Jul;58(1):76-84. doi: 10.1016/j.toxicon.2011.05.006. Epub 2011 May 18. PMID: 21619891
Inhibition of gap-junctional intercellular communication and activation of mitogen-activated protein kinases by cyanobacterial extracts--indications of novel tumor-promoting cyanotoxins?	Bláha et al.	Toxicon. 2010 Jan;55(1):126-34. doi: 10.1016/j.toxicon.2009.07.009. Epub 2009 Jul 18. PMID: 19619572
Comparative study of cyanotoxins affecting cytoskeletal and chromatin structures in CHO-K1 cells	Gácsi et al.	Toxicol In Vitro. 2009 Jun;23(4):710-8. doi: 10.1016/j.tiv.2009.02.006. Epub 2009 Feb 27. PMID: 19250963
Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin	Froscio et al.	J Toxicol Environ Health A. 2009;72(5):345-9. doi: 10.1080/15287390802529906. PMID: 19184750
Toxicity of cylindrospermopsin, and other apparent metabolites from Cylindrospermopsis raciborskii and Aphanizomenon ovalisporum, to the zebrafish (Danio rerio) embryo	Berry et al.	Toxicon. 2009 Feb;53(2):289-99. doi: 10.1016/j.toxicon.2008.11.016. Epub 2008 Dec 6. PMID: 19087885
Induction of p53-regulated gene expression in human cell lines exposed to the cyanobacterial toxin cylindrospermopsin	Bain et al.	J Toxicol Environ Health A. 2007 Oct;70(19):1687-93. PMID: 17763087
Studies of the comparative in vitro toxicology of the cyanobacterial metabolite deoxycylindrospermopsin	Neumann et al.	J Toxicol Environ Health A. 2007 Oct;70(19):1679-86. PMID: 17763086
Toxicity and uptake mechanism of cylindrospermopsin and lophyrotomin in primary rat hepatocytes	Chong et al.	Toxicon. 2002 Feb;40(2):205-11. PMID: 11689242
Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria—experimental evidence	Torokne et al.	Environ Toxicol. 2001;16(6):512-6. PMID: 11769249