EPA’s Guidelines for Cyanotoxins

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US Environmental Protection Agency
Office of Water/Office of Science and Technology
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Presentation Overview

• Describe public health guidelines in place

• Discuss the toxicity assessment done for the three cyanotoxins listed in CCL

• Discuss the development of the Health Advisories

• Discuss current efforts to develop Ambient Water Quality Criteria for Recreational Exposures

• Present an overview of the evaluation of potential bioaccumulation of cyanotoxins in fish.

• Opportunity for Questions
Drinking Water Guidelines for Cyanotoxins

- No federal regulations for cyanobacteria/cyanotoxins in drinking water in the U.S.

<table>
<thead>
<tr>
<th>Authority/Country/State</th>
<th>Microcystin</th>
<th>CYL</th>
<th>Anatoxin-a</th>
<th>Saxitoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>World Health Organization (WHO), 2003</td>
<td>1 μg/L MC-LR</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Health Canada, 2002</td>
<td>1.5 μg/L MC –LR</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brazil, 2005</td>
<td>1 μg/L MC-LR</td>
<td>15 μg/L</td>
<td>-</td>
<td>3 μg/L</td>
</tr>
<tr>
<td>Australia, 2009</td>
<td>1.3 μg/L MC-LR TE</td>
<td>1 μg/L</td>
<td>3 μg/L</td>
<td>3 μg/L</td>
</tr>
<tr>
<td>New Zealand, 2009</td>
<td>1 μg/L MC-LR TE</td>
<td>1 μg/L</td>
<td>6 μg/L</td>
<td>-3 μg/L</td>
</tr>
<tr>
<td>Denmark</td>
<td>1 μg/L</td>
<td>-</td>
<td>6 μg/L</td>
<td>-</td>
</tr>
<tr>
<td>Singapore, Poland, Norway, China, Netherlands, Korea, Japan, Italy, France, Germany, Finland, Czech Republic</td>
<td>1 μg/L MC-LR</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ohio, 2015</td>
<td>0.3 μg/L bottle-fed infants and pre-school age children</td>
<td>0.7 μg/L bottle-fed infants and pre-school age children</td>
<td>20 μg/L</td>
<td>0.2 μg/L</td>
</tr>
<tr>
<td>Oregon</td>
<td>1 μg/L MC-LR</td>
<td>1 μg/L</td>
<td>3 μg/L</td>
<td>3 μg/L</td>
</tr>
<tr>
<td>Minnesota</td>
<td>0.04 μg/L MC-LR</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Recreational Water (RW) Guidelines for Cyanotoxins

- No federal regulations or guidelines for cyanobacteria/cyanotoxins in RW in the U.S.

<table>
<thead>
<tr>
<th>Authority/State</th>
<th>Recreational Water Guidance/Action Level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WHO</strong></td>
<td><strong>Relative Probability of Acute Health Effects</strong></td>
</tr>
<tr>
<td></td>
<td>Cyanobacteria (cells/mL)</td>
</tr>
<tr>
<td>Low</td>
<td>&lt; 20,000</td>
</tr>
<tr>
<td>Moderate</td>
<td>20,000-100,000                                            Microcystin-LR (µg/L) 20-20                  Chlorophyll-a (µg/L) 10-50</td>
</tr>
<tr>
<td>High</td>
<td>100,000-10,000,000                                          &gt;2,000                            &gt;5,000</td>
</tr>
<tr>
<td>Very High</td>
<td>&gt; 10,000,000</td>
</tr>
<tr>
<td></td>
<td><strong>Cyanobacteria (cells/mL)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Microcystin-LR (µg/L)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Chlorophyll-a (µg/L)</strong></td>
</tr>
<tr>
<td>California</td>
<td>Microcystin: 0.8 µg/L; Anatoxin-a: 90 µg/L; Cylindrospermopsin: 4 µg/L</td>
</tr>
<tr>
<td>Illinois</td>
<td>Microcystin-LR concentration results approach or exceed 10 µg/L</td>
</tr>
<tr>
<td>Indiana</td>
<td>L1: very low/no risk &lt; 4 µg/L MC-LR; L2: low to moderate risk 4 to 20 µg/L MC-LR</td>
</tr>
<tr>
<td></td>
<td>L3: serious risk &gt; 20 µg/L microcystin-LR; Cylindrospermopsin: 5 ppb</td>
</tr>
<tr>
<td>Iowa, Nebraska, Oklahoma, Texas</td>
<td>Microcystin ≥ 20 µg/L</td>
</tr>
<tr>
<td>Kansas</td>
<td>HA: &gt;4 µg/L to &lt;20 µg/L for MC or &gt; 20,000 cell/mL to &lt;100,000 cell/mL cyanobacteria cells</td>
</tr>
<tr>
<td></td>
<td>HW: &gt; 20 µg/L or &gt; 100,000 cell/mL cyanobacterial cell counts and visible scum present</td>
</tr>
<tr>
<td>Kentucky (Louisville District)</td>
<td>Advisory: &gt;20,000 cells/mL of cyanobacteria cells; Caution: &gt; 100,000 cells/mL of cyanobacteria cells</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>14 µg/L for microcystin-LR and ≥ 70,000 cells/mL for cyanobacteria cell counts</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>&gt;50% of cell counts from toxigenic cyanobacteria</td>
</tr>
<tr>
<td>North Carolina</td>
<td>Visible discoloration of the water or a surface scum may be considered for microcystin testing</td>
</tr>
<tr>
<td>Ohio</td>
<td>Microcystin-LR: PHA: 6 µg/L; NCA: 20 µg/L; Anatoxin-a: PHA: 80 µg/L; NCA: 300 µg/L</td>
</tr>
<tr>
<td></td>
<td>Saxitoxin: PHA: 0.8 µg/L; NCA: 3 µg/L; Cylindrospermopsin: PHA: 5 µg/L; NCA: 20 µg/L</td>
</tr>
<tr>
<td>Oregon</td>
<td>Microcystin: 10 µg/L; Anatoxin-a: 20 µg/L; Cylindrospermopsin: 6 µg/L; Saxitoxin: 100 µg/L</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>Visible cyanobacteria scum or mat and/or cyanobacteria cell count &gt; 70,000 cells/mL and/or ≥14 µg/L of MC-LR</td>
</tr>
<tr>
<td>Vermont</td>
<td>4,000 cells/mL cyanobacteria cell counts or ≥ 6µg/L MC-LR and the visible presence of cyanobacterial scum; Anatoxin-a ≥ 10 µg/L</td>
</tr>
<tr>
<td>Virginia</td>
<td>Microcystin provisional action level: 6µg/L</td>
</tr>
<tr>
<td>Washington</td>
<td>Microcystin-LR: 6 µg/L; Anatoxin-a: 1 µg/L; Cylindrospermopsin: 4.5 µg/L; Saxitoxin: 75 µg/L</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>&gt; 100,000 cells/mL or scum layer</td>
</tr>
</tbody>
</table>
Cyanotoxins Toxicity Assessment

- Microcystin-LR
- Cylindrospermopsin
- Anatoxin-a
Health Effects Assessment: Microcystins

• Most studied and widespread cyanobacterial toxin (microcystin-LR).
• More than 100 congeners exist.
• The toxicological database is almost exclusively limited to data on the -LR congener.

Noncancer Effects
• Human data suggest that the liver is the target organ of toxicity
• Studies in laboratory animals have demonstrated toxicity in the liver, kidney, and testes
  • Acute and short term studies, and sub-chronic studies
    • Liver, kidney, reproductive, and developmental effects
  • Chronic studies
    • Limited and have not reported significant effects

Cancer Effects
• Human epidemiological studies have reported an association between consumption of drinking water with cyanobacteria and microcystins and liver or colon cancer in certain areas of China.
• No chronic cancer bioassays designed to evaluate dose-response for the tumorigenicity of microcystins following lifetime exposures are available.
• Applying the EPA 2005 Guidelines for Carcinogen Risk Assessment, there is inadequate information to assess the carcinogenic potential of microcystins.
Health Effects Assessment: Cylindrospermopsin

Noncancer Effects

• Human data on oral toxicity of cylindrospermopsin suggests liver and kidney as the target organs.

• Animal laboratory studies focused on hepatic and renal toxicity
  • Acute, short-term, and subchronic studies demonstrate the liver and kidney as target organs.
  • No chronic studies were identified.

Cancer

• Applying the 2005 EPA Guidelines for Carcinogen Risk Assessment, there is *inadequate information to assess the carcinogenic potential* of cylindrospermopsin.
  • No human or chronic cancer bioassays in laboratory animals are available
Health Effects Assessment: Anatoxin-a

Noncancer Effects
• Human data on oral toxicity suggests the nervous system as the target organ.
• Acute and short-term animal laboratory studies are limited.
• No chronic studies were identified.
• Not enough information on sensitive endpoints and associated dose-response relationships to develop an RfD.

Cancer
• There are no cancer, genotoxicity, acute or chronic exposure studies on anatoxin-a, thus there is inadequate information to assess carcinogenic potential.
DW Health Advisories for Cyanotoxins

- Microcystins
- Cylindrospermopsin
EPA Health Advisories

• Informal technical guidance, non-regulatory concentrations estimated for specific exposure durations:
  • Short term exposures: one-day and ten-day (children)
    – One-day HA assumes a single acute exposure; derived from a study of less than 7 days’ duration
    – Ten-day HA assumes a limited period of one to two weeks exposure; derived from a study of less than 30-days duration.
  • Chronic Exposures: lifetime (for adults)
    – Derived from a chronic study of 2 years duration, but subchronic studies may be used by adjusting the uncertainty factor employed in the calculation.
    – Updated BW represents the mean weight for adults ages 21 and older. EPA updated the default DWI to 2.5 L/d, rounded from 2.546 L/d, based on values in Table 3-33 in the EPA’s Exposure Factors Handbook.
• Carcinogenic
• Inference:
  • Concentration in drinking water that is not expected to cause any adverse non carcinogenic effects for a specific exposure period.
Children’s Exposure to Cyanotoxins

- Bottle-fed infants consume large amounts of drinking water compared to their body weight.
- Exposure to children < 12 months is 5 times higher than for adults > 21 years old, on a body-weight basis.
- At 6 years and older, exposure on a body-weight basis is similar to that of an adult.
Cyanotoxins Health Advisories Development

- 2012 – Joint effort with Health Canada
- 2013 - Literature Review and Health Effects Support Documents (HESD) for microcystin, cylindrospermopsin and anatoxin-a development
  - Comprehensive review of the health effects information.
  - Provides the health effects basis for the development of HAs.
- 2014 -2015 External Peer Reviews HESDs for Anatoxin-a, Cylindrospermopsin and Microcystins
  - Peer reviewers affirmed there is inadequate information to develop an HA for anatoxin-a
  - Peer reviewers confirmed there is adequate information to develop HAs for microcystins and cylindrospermopsin
- 2015 –Development of HA for Microcystins and Cylindrospermopsin
- June 17th, 2015 – HAs Published
EPA Drinking Water HAs for Microcystins

- **Stressor:** microcystin-LR, considered a surrogate for all microcystins
  - Data are most complete
  - LR is the same or more toxic than other congeners, based on available data
- **Exposure pathway:** oral ingestion of drinking water
- **Key Study Selected:** Heinze, 1999; 28 day drinking water study in rats
- **Most sensitive endpoint:** liver toxicity
  - Increase in liver weight and in liver enzymes
- **POD:** 50 μg/kg/day (LOAEL)
- **Exposed life stage and population:** infants and adults
- **Exposure duration:** 10-day value
  - Short term exposure is more consistent with expected exposure pattern
  - No lifetime or carcinogenic value derived

- **Ten-day HA for bottle-fed infants**
  \[ HA_{10\text{ day}} = \frac{50 \ \mu g/Kg/d}{1000 \times 0.15 \ L/kg/day} = 0.3 \ \mu g/L \]

- **Ten-day HA for adult**
  \[ HA_{10\text{ day}} = \frac{50 \ \mu g/Kg/d}{1000 \times 0.03 \ L/kg/day} = 1.6 \ \mu g/L \]

  - **LOAEL** = 50 μg/kg/day
  - **UF** = 1000: intraspecies:10; interspecies: 10; LOAEL to NOAEL: 10^{0.5}; database: 10^{0.5}
  - **DWI/BW** = 0.15L/kg/day normalized drinking water intakes per unit body weight over the first year of life
    0.03 L/kg/day based on adult defaults of 2.5 L/day and 80 kg
EPA Microcystins Health Advisory by Age Group

- **Bottle fed infants up to school age children:** 0.3 µg/L
- **School-age children and adults:** 1.6 µg/L
EPA Drinking Water HAs for Cylindrospermopsin

- **Stressor:** cylindrospermopsin
- **Exposure pathway:** oral ingestion of drinking water (by gavage)
- **Key Study Selected:** Humpage and Falconer (2002, 2003); 11 weeks drinking water study in mice
- **Most sensitive endpoint:** kidney damage
  - Increased weight of kidney and decreased urinary protein
- **Exposed life stage and population:** children and adults
- **Exposure duration:** 10-day value
  - No lifetime or carcinogenic value
- **POD:** 30 μg/kg/day (NOAEL)
- **Exposed life stage and population:** infants and adults
- **Exposure duration:** 10-day value
  - Short term exposure is more consistent with expected exposure pattern
  - No lifetime or carcinogenic value derived

- **Ten-day HA for bottle-fed infants**
  \[
  HA_{10\text{-day}} = \frac{30 \text{ μg/Kg/d}}{300 \times 0.15\text{L/kg/day}} = 0.7 \text{ μg/L}
  \]

- **Ten-day HA for adult**
  \[
  HA_{10\text{-day}} = \frac{30 \text{ μg/Kg/d}}{300 \times 0.03\text{L/kg/day}} = 3 \text{ μg/L}
  \]

- **LOAEL** = 30 μg/kg/day
- **UF** = 300 (intraspecies:10; interspecies: 10; database: \(10^{0.5}\))
- **DWI/BW** = 0.15L/kg/day normalized drinking water intakes per unit body weight over the first year of life
  
  0.03 L/kg/day based on adult defaults of 2.5 L/day and 80 kg
EPA Cylindrospermopsin Health Advisory by Age Group

- Bottle fed infants up to school age children: 0.7 µg/L
- School-age children and adults: 3 µg/L
## Difference among EPA and WHO GV for MCs

<table>
<thead>
<tr>
<th></th>
<th>Principal Study</th>
<th>Duration /Route</th>
<th>Dose (µg/kg-d)</th>
<th>Endpoint</th>
<th>Point of Departure (µg/kg-d)</th>
<th>Uncertainty Factors</th>
<th>TDI (µg/kg-d)</th>
<th>Guideline Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO (1999) Provisional GV for MC-LR</td>
<td>Fawell et al. (1994)</td>
<td>13 weeks; gavage; MC-LR</td>
<td>0, 40, 200, and 1000</td>
<td>Minimal/ light chronic inflammation; increased serum enzymes</td>
<td>NOAEL= 40</td>
<td>10-interspecies 10-intraspecies 3-LOAEL to NOAEL total = 1000</td>
<td>0.04</td>
<td>1 µg/L provisional for MC-LR Applies to a Lifetime Exposure *WHO applied an allocation factor of 0.80 to account for the proportion of daily exposure arising from drinking water</td>
</tr>
<tr>
<td>U.S.EPA GV for MCs</td>
<td>Heinze, 1999</td>
<td>28 day; drinking water; purified extract MC-LR</td>
<td>0, 50, 150 µg/kg-d</td>
<td>Increased liver weight, increased serum enzymes; degenerative and necrotic hepatocytes with hemorrhage</td>
<td>LOAEL = 50</td>
<td>10-interspecies 10-intraspecies 3-LOAEL to NOAEL total = 1000</td>
<td>0.05</td>
<td>0.3 µg/L for infants and 1.6 µg/L for adults for MCs Applies to Short-term (10-day) Exposures</td>
</tr>
</tbody>
</table>
Data Gaps Identified

- The toxicity of microcystins to the male reproductive system after sub-acute to chronic oral exposure.
- The toxicity of microcystins to the female reproductive tissues and those of offspring following oral exposure.
- The relative potencies of other microcystin congeners when compared to microcystin-LR.
- The adverse effects of inhalation and/or dermal exposures to cyanotoxins.
- The carcinogenic potential of cyanotoxins.
- Potential health risks from exposure to mixtures of cyanotoxins.
- Bioconcentration and bioaccumulation of cyanotoxins in aquatic food webs.
 Ambient Water Quality Criteria (AWQC) Development for Recreational Exposures

• Clean Water Act §304(a) recommends recreational Ambient Water Quality Criteria (AWQC) values protective of human health given a primary contact recreational exposure scenario.

• To provide guidance to ensure safety for recreational exposures to cyanotoxins, EPA is developing recreational AWQC recommendations for the cyanotoxins microcystin toxins and cylindrospermopsin.

• The AWQC will focus on exposure from primary contact recreation activities such as swimming where immersion and incidental ingestion of ambient water are likely.
  – Dermal and inhalation exposures associated with primary contact recreation will be considered if data are sufficient.
  – Consumption of fish and shellfish will not be considered in the assessments.

• Expected Draft: 2016
Evaluation of Potential Bioaccumulation of Cyanotoxins in Fish

- Microcystin-LR
- Cylindrospermopsin
- Anatoxin-a

Steps for deriving BAFs for cyanotoxins:
- Collect and review all relevant bioaccumulation data for the cyanotoxins for review for adequacy.
- Calculate individual toxin-specific BAF for each trophic level.

BAF Derivation equation used by EPA in the 2000 Human Health Methodology

\[
\text{BAF} = \frac{C_t}{C_w}
\]

where:
- \(C_t\) = total concentration of the chemical in wet tissues
- \(C_w\) = total concentration of chemical in water
# Preliminary Summary of MC Concentrations in Fish

<table>
<thead>
<tr>
<th>Trophic Level</th>
<th>Species/tissue</th>
<th>Concentration of Microcystins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Shrimp</td>
<td>Whole Muscle Hepatopancreas</td>
<td>0.051-0.114 µg/g ww&lt;br&gt;0.004-0.006 µg/g ww&lt;br&gt;55 µg/g ww</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crayfish Whole</td>
</tr>
<tr>
<td></td>
<td>Tilapia (rendalli and Oreochrois niloticus)</td>
<td>Muscle Liver Viscera</td>
<td>0.002-0.337 µg/g ww&lt;br&gt;0.092- 31.1µg/g ww&lt;br&gt;0-71.6 µg/g ww</td>
</tr>
<tr>
<td>3</td>
<td>Carp (Cyprinus carpio)</td>
<td>Muscle Liver Hepatopancreas</td>
<td>0.05 - 0.157 µg/g fresh ww&lt;br&gt;0.094-0.867 µg/g ww&lt;br&gt;0.123 - 0.261 µg/g ww</td>
</tr>
<tr>
<td></td>
<td>Silver carp (Hypophtalmichtys sp.)</td>
<td>Muscle Liver Intestine</td>
<td>1.77 - 1.81 µg/g dw&lt;br&gt;7.77 µg/g dw&lt;br&gt;22 µg/g dw</td>
</tr>
<tr>
<td></td>
<td>Crab</td>
<td>Muscle</td>
<td>0.103 – 0.4 µg/g ww</td>
</tr>
<tr>
<td></td>
<td>Silverside fish (Odontesthes bonariensis)</td>
<td>Muscle Liver Gill</td>
<td>0.05/0.34 µg/g ww&lt;br&gt;0.16/1.01 µg/g ww&lt;br&gt;0.03/0.10 µg/g ww</td>
</tr>
<tr>
<td></td>
<td>Yellow perch (Perca flavescens)</td>
<td>Muscle Liver</td>
<td>0.00012-0.004 µg/g ww&lt;br&gt;0.017-1.182 µg/g ww</td>
</tr>
<tr>
<td></td>
<td>Catfish (Ictalarus punctatus)</td>
<td>Muscle Liver</td>
<td>0.21µg/g ww&lt;br&gt;0.123 – 0.250 µg/g ww</td>
</tr>
<tr>
<td></td>
<td>Largemouth black bass (Micropterus salmoides)</td>
<td>Muscle Liver</td>
<td>0.32 µg/g&lt;br&gt;0.21 µg/g&lt;br&gt;0.32µg/g&lt;br&gt;0.27 µg/g</td>
</tr>
<tr>
<td></td>
<td>White crappie (Pomoxis annularis)</td>
<td>Muscle Liver</td>
<td>0.21 µg/g&lt;br&gt;0.27 µg/g</td>
</tr>
</tbody>
</table>

*Trophic level assignments based on the Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)

* Exposure routes from feeding on MC’s scums in tanks or natural routes in lake.
### Preliminary Summary of CYL Concentrations in Fish

<table>
<thead>
<tr>
<th>Trophic Level</th>
<th>Species/tissue</th>
<th>Concentration of Microcystins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Crayfish</td>
<td>Muscle tissue</td>
<td>Saker and Eaglesham, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatopancreas</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rainbow fish</td>
<td>Viscera</td>
<td>Saker and Eaglesham, 1999</td>
</tr>
</tbody>
</table>

* *Trophic level assignments based on the Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)*
* *Exposure routes from feeding on MC’s scums in tanks or natural routes in lake.*

### Preliminary Summary of Anatoxin-a Concentrations in Fish

<table>
<thead>
<tr>
<th>Trophic Level</th>
<th>Species/tissue</th>
<th>Concentration of Microcystins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Carp (Cyprinus carpio)</td>
<td>Whole</td>
<td>Osswald et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.005 – 0.073 µg/g fresh ww</td>
<td></td>
</tr>
</tbody>
</table>

* *Trophic level assignments based on the Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)*
* *Exposure routes from feeding on MC’s scums in tanks or natural routes in lake.*
Findings...so far

- Exposure to high levels of cell-bound toxin are more of a concern for accumulation in fish than when compared with dissolved toxin. (Kinnear, 2010 and GWA Nyakairu, 2010)
- MC’s levels in edible fish and shellfish are highly variable depending on trophic level and fish organs and tissues. (Ibelings and Havens, 2008, Zhang et al, 2009)
  - Concentrations are higher in liver > gut > kidneys and gonads > muscle tissue
  - Concentrations are higher > phytoplanktivorous > omnivorous > carnivorous fish
- Different aquatic animals may have different metabolism routes and accumulation pattern of MCs.
- Rather than biomagnification, biodilution seems to occur in the foodweb with toxins being subject to degradation and excretion at every level. (Ibelings and Havens, 2008).
Contact Information

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EPA’s CyanoHABs Website
www.epa.gov/cyanohabs