Cyanobacterial toxins: Cylindrospermopsin

Background document for development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments

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Preface

Information on cyanobacterial toxins, including cylindrospermopsins, is comprehensively reviewed in a recent volume to be published by the World Health Organization, “Toxic Cyanobacteria in Water” (TCiW; Chorus & Welker, in press). This covers chemical properties of the toxins and information on the cyanobacteria producing them as well as guidance on assessing the risks of their occurrence, monitoring and management. In contrast, this background document focuses on reviewing the toxicological information available for guideline derivation and the considerations for deriving the guideline values for microcystins in water. Sections 1-3 and 8 are largely summaries of respective chapters in TCiW and references to original studies can be found therein.

To be written by WHO Secretariat

Acknowledgements

The first draft of the background document on cylindrospermopsin in drinking-water for the development of the WHO Guidelines for Drinking-water Quality was prepared by Dr Andrew Humpage of the South Australian Water Corporation (Retired).......
Abbreviations used in the text

bw  body weight
C   daily consumption of drinking water
CYN cylindrospermopsin
CYP450 cytochrome P450
dw  dry weight
ELISA enzyme-linked immunosorbent assay
GD  gestational day
GSH glutathione
i.p. intraperitoneal
LC-MS liquid chromatography – mass spectrometry
LOAEL lowest-observed-adverse-affect level
NOAEL no-observed-adverse-affect level
PoD point of departure
UF  uncertainty factor
WI  water intake
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1.0 EXECUTIVE SUMMARY

2.0 GENERAL DESCRIPTION

2.1 Identity

Cylindrospermopsin (CYN) is a naturally occurring alkaloid produced by a number of genera of cyanobacteria. Naturally occurring structural variants are 7-epi-CYN and 7-deoxy-CYN. Two further variants have been reported from a strain of *C. raciborskii* originating from Thailand, 7-deoxy-desulfo-CYN and 7-deoxy-desulfo-12-acetyl-CYN, but it is not clear whether these are actual congeners or degradation products.

All CYN variants consist of a tricyclic guanidino moiety linked via a hydroxylated bridging carbon (C7) to uracil. The uracil moiety is required for toxicity.

![Molecular structure of common cylindrospermopsins.](image)

2.2 Physicochemical Properties

CYN molecules are zwitterionic (i.e., a dipolar ion with localized positive and negative charges) and hence very hydrophilic. Known physico-chemical properties are summarized in Table 1. Other physico-chemical properties of CYN such as vapor pressure, boiling and melting point, soil adsorption coefficient (Koc), or Henry’s Law constant have not been determined.

<table>
<thead>
<tr>
<th>Property</th>
<th>cylindrospermopsin</th>
<th>7-deoxy-cylindrospermopsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASRN&lt;sup&gt;1&lt;/sup&gt;</td>
<td>143545-90-8</td>
<td>N/A</td>
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<tr>
<td>Chemical Formula</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;S</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;S</td>
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<td>399.429</td>
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<tr>
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<tr>
<td>Kow&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>-1.4</td>
</tr>
<tr>
<td>Koc</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Solubility in Water</td>
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<td>High</td>
</tr>
</tbody>
</table>

<sup>1</sup> Chemical Abstracts Service Registry Number
<sup>2</sup> Molecular Weight
<sup>3</sup> logP computation with XLogP3 (Cheng et al., 2007)
2.3 Organoleptic properties

While none of the known cyanobacterial toxins have been shown to affect the taste or odour of water, some cyanobacterial species produce other compounds such as geosmin and methyl-isoborneol that do affect taste or odour of water, thus indicating the presence of cyanobacteria in raw water. However, as this applies only to some strains of some species, the absence of tastes or odours is not a reliable indicator for the absence of cyanotoxins. Taste or odour thresholds in water are 0.004 ppb for geosmin and 0.006 ppb for methyl-isoborneol (TCiW, Kaloudis, in press).

2.4 Major uses and sources

CYN’s occur naturally (although high concentrations are typical for waterbodies influenced by human activity, i.e. effluents from wastewater or run-off from agricultural land which introduce nutrients that fertilise the growth of phototrophic organisms, including cyanobacteria. There are no known commercial applications of CYNs. The CYNs are produced by various species, primarily within the Nostocales (Anabaena, Aphanizomenon, Cylindrospermopsis, Raphidiopsis, Umezakia) and Oscillatoriales (Lyngbya, Oscillatoria), many of the latter being primarily benthic (i.e. growing on sediments or other submerged surfaces). Not all strains within potentially toxigenic species produce CYN. Interestingly, the distribution of CYN producing strains of some species follows a geographic pattern which has changed in recent decades: While Cylindrospermopsis raciborskii is a major producer in Australia, New Zealand and Asia, strains of this species originating from Europe, the Americas, and Africa generally lack the genes for the biosynthesis of CYN. In Europe, CYN production is largely confined to Aphanizomenon sp. and Dolichospermum sp.

The relative share of the individual CYN structural variants is variable among producing strains. While in most strains the highest share is CYN, in rare cases the share of deoxy-CYN can be highest. Where CYNs occur, CYN is found dominant in most field samples but occasionally deoxy-CYN is dominant, likely due to the clonal composition of the bloom. The CYN production or content, respectively, is highly variable between individual strains, ranging from some 10 µg up to 10 mg/g dry weight across all producing species and geographical regions. Cell quotas of CYN reported in planktonic species range from <10 to 190 fg/cell and 0.3 to 3.5 µg/mm³ of biovolume.

The biosynthesis of CYN has been largely elucidated. It starts with an amidinotransferase and is completed by non-ribosomal peptide synthetases, polyketide synthases and tailoring enzymes, the genes of which are organized in a large gene cluster (cyrA-O, ca. 44 kbp) that is known from several species (C. raciborskii, Chrysosporum ovalisporum, Aphanizomenon sp., Oscillatoria sp., Raphidiopsis curvata). The regulation of gene transcription and hence CYN production is thought to be coupled to the cells’ nitrogen metabolism. The presence of the biosynthesis cluster in the genome of a particular strain is a strong indicator of CYN production, i.e., CYN is synthesized constitutively by toxigenic strains.

An intermediate of CYN biosynthesis, guanidinoacetate, is known to be toxic and has been found to accumulate in individual CYN-producing strains. It may contribute to the total toxicity.

Cyanobacteria producing CYN are known primarily from freshwater environments, including lakes and reservoirs used for drinking-water production or for recreation. Therefore, exposure
to CYN is primarily through contact with contaminated water, either drinking-water or during recreational activities.

For more details on CYN producing organisms and biosynthesis see TCiW (Humpage & Fastner, in press).

3.0 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

CYN is not volatile and hence exposure via inhalation is possible only through aerosols. It could also occur through cyanobacterial cells carried in spray, e.g. during storms or in the wake of a power boat. No information on exposure via this route was found nor were data on concentrations in aerosols.

3.2 Food

CYN may bioaccumulate in a range of aquatic species potentially consumed, such as fish or shellfish. It is not degraded by boiling (100 °C for 5 min), hence cooking is not likely to reduce its concentration in food. CYN concentrations reported vary widely between types of fish and shellfish as well as between organs and tissues. For fish muscle, concentrations have been reported in the low range of µg/kg fresh weight while in crayfish muscle they ranged up to a few 100 µg CYN/kg fresh weight and in freshwater mussels up to several hundred µg CYN/kg fresh weight. Depuration has been observed to set in readily after exposure to CYN. Data on food items potentially contaminated with CYN are scarce and systematic monitoring is not in place. CYN has not been reported from algal dietary supplements. No field data are available on CYN occurrence in crops; however, in experimental settings, transfer of CYN from root to leaves was observed.

For more details on CYN in food and dietary supplements see TCiW (Ibelings & Chorus, in press).

3.3 Water

In many settings the major water-borne route of human exposure to CYN will be the consumption of drinking-water, most likely where it is produced from surface waters with insufficiently effective or non-existent treatment. A further exposure route – important in some settings – is the recreational use of lakes and rivers. Depending on the seasonal patterns of cyanobacterial blooms and water body use, patterns of exposure may be episodic.

CYN has been detected in surface waters around the globe. The in situ CYN concentration is largely coupled to the abundance of producing organisms and highest CYN concentrations expectedly occur during peak water blooms. However, in contrast to other cyanobacterial toxins, in particular microcystins, CYN occurs in surface water in the dissolved fraction in relatively high shares, up to 90% of total CYN, through the release of CYN from viable cells. In laboratory experiments, the release of CYN and the relative share of dissolved CYN of the total CYN pool was found to reach levels exceeding 50% but the mechanisms and the regulation of this process have not yet been revealed. In combination with the persistence of CYN, CYN concentrations in a water body may remain high long after the producing species has disappeared (i.e. at low water temperature for weeks). To date, no heterotrophic bacteria
capable of degrading CYN have been isolated and degradation experiments with bacterial consortia showed CYN to persist microbial degradation for several weeks. Hence, while the cell-bound CYN concentration may be comparatively low, the concentration of dissolved CYN can be relatively high, well exceeding the guideline values. This is highly relevant for drinking-water treatment procedures (see below).

The highest levels of CYN reported from the environment are a few hundred µg/L. In most surveys, CYN concentrations in surface waters, in particular in open water samples, are in the low µg/L range (<10 µg/L) and only occasionally up to 800 µg/L. This is considerably lower than for other cyanotoxins, particularly microcystins – probably because the main producing organisms (Cylindrospermopsis, Aphanizomenon, Dolichospermum, Chrysosporum) do not tend to form surface blooms or massive scums leading to an increase in concentrations by orders of magnitudes (as is observed for microcystins, particularly in Microcystis blooms). Further, globally CYN is detected less frequently than microcystins, presumably due to the less frequent occurrence of CYN producing strains in surface waters. However, the number of extensive surveys including all known cyanotoxins is low and on a regional or local scale, CYN may well occur more frequently. This seems to be the case particularly in tropical and subtropical regions but also, e.g., in northern Germany.

CYN concentrations in finished drinking water up to 2.2 µg/L have been reported, with the number of investigations of CYN in drinking water being quite limited. A breakthrough of CYN from raw water into finished drinking-water is a relevant scenario especially because of the potentially high shares of dissolved CYN that can efficiently pass filtration or flocculation steps in the treatment process.

Recreational activity in surface waters with cyanobacterial blooms causes relevant exposure primarily through unintentional swallowing of water, particularly where surface blooms or scums accumulate. While high levels of CYN in scum have not been reported, some CYN-producing cyanobacteria (e.g. Aphanizomenon and Dolichospermum) can form scums, rendering this route of exposure possible.

For more details on CYN occurrence in the environment and drinking water see TCiW (Humpage & Fastner, in press).

3.4 Estimated total exposure and relative contribution of drinking-water

Drinking-water is the most likely source of exposure to MC where surface water sources are used. However, this assumption serves as starting point and country- or region-specific circumstances should take into account the contribution of foods on exposure, which in certain settings may be a significant source of exposure. Likewise, recreational activities in lakes with cyanobacterial blooms may expose individuals to high concentrations of MC. Significant dermal or inhalational uptake of CYN during recreational exposure is unlikely, leaving the oral route as the main route of concern.

Exposure patterns and durations are strongly influenced by region and lifestyle. Estimating total exposure or the relative contribution of particular exposure routes (e.g., food, drinking-water) requires specific analyses of concentrations in respective media in a given setting. Exposure may be elevated in tropical regions where blooms of CYN-producing cyanobacteria may persist for extended periods.
4.0 KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

4.1 Absorption

Absorption information for CYN in humans was not identified. CYN is absorbed from the gut of mice as inferred by effects on the liver and other tissues following oral dosing (Shaw et al., 1999, 2000, 2001; Humpage & Falconer, 2003), as well as studies in fish using immunohistochemical techniques that reported immunopositive results in various organs (Guzman-Guillén, et al., 2014). However, there are no studies that have determined the rate or efficiency of CYN uptake via this or any other route. Since CYN is a hydrophilic molecule, its intestinal absorption as well as the uptake into other cell types including hepatocytes need to be mediated by active transport systems, such as the bile acid transport system, e.g., cholate and taurocholate (Chong et al., 2002). However, due to the small size of the molecule, a limited passive diffusion through biological membranes is expected, supported by some results obtained in in vitro systems, such as cytotoxic effects observed in a cell line not expressing bile acid transport system (Chong et al., 2002) and the energy independent slow and progressive uptake of purified CYN into renal (Vero) cells (Frosio et al., 2009). The permeation across the Caco-2 cell monolayer was limited but time and concentration dependent (Fernández et al., 2014).

4.2 Distribution

Systemic distribution of CYN following oral exposure of laboratory animals to the purified toxin is implied by the range of tissues affected including the liver, kidneys, heart and thymus (Terao et al., 1994; Humpage & Falconer, 2003). The only mammalian study investigating tissue distribution of CYN in a quantitative manner used i.p. injection (Norris et al., 2001). Six hours after i.p. injection of a median lethal dose of radiolabelled CYN (0.2 mg/kg), radiolabel was detected in all examined tissues (liver, kidney, heart, lung, spleen, blood and bile), with average radiolabel recoveries of 20% in the liver and 4% in the kidney. By 5-6 days, these recoveries were 3% and 0.2%, respectively, with high variation between animals. A week after dosing, about 2% of the label was still detectable in the liver (Norris et al., 2001). There are some studies in fish: studies on the distribution of cylindrospermopsin (200 µg pure CYN/Kg body weight (bw) administered by i.p. or by gavage) in fish (Oreochromis niloticus) using immunohistochemical techniques have found after 5 days of exposure immunopositive results in the liver, followed by the kidney, intestines, and gills (Guzman-Guillén, et al., 2014).

4.3 Metabolism

Laboratory animal studies provide some indirect evidence that cytochrome P450 enzymes are involved in the metabolic activation of CYN to a more rapidly cytotoxic compound, both in vitro (Frosio et al., 2003) and in vivo (Shaw et al., 2001; Norris et al., 2002). This hypothesis is supported by the co-localization of hepatic toxicity mainly in the periacinar region, where substantial CYP450-mediated xenobiotic metabolism occurs and by the in vivo and in vitro effects of inducers and inhibitors of CYP450 on CYN toxicity (Humpage et al., 2005; Funari & Testai, 2008; Bazin et al., 2012). However, studies with metabolically-competent liver HepaRG cells and rat and human liver tissue failed to detect phase I metabolites after 24 h incubation of a relatively low CYN concentration (72 nM), although co-treatment with a known CYP3A4 inhibitor significantly decreased cytotoxicity (Kittler et al., 2016).
Phase II metabolism was not investigated. The GSH depletion, observed after CYN oral administration to rats, leads to the hypothesis that CYN can be further metabolized by GSH conjugation (Runnegar et al. 1995), but this possibility was not yet confirmed or excluded.

4.4 Elimination

Information on the elimination or excretion of CYN in exposed humans was not identified and no laboratory animal studies have quantified the excretion of CYN following oral dosing. Six hours after a single i.p. injection of 0.2 mg/kg radiolabelled CYN, average excretion into the urine and faeces was 48% and 12%, respectively, of the original dose. In a separate experiment, these values were 66% and 5.7%, respectively; the majority occurred during the first 0–12 h since by 24 hrs, these values were 68% and 8.5%, respectively. High variability was seen in the total amount and pattern excreted, with a generally inverse relationship observed between urinary and faecal excretion in different animals (Norris et al., 2001). About 70% of the excreted material, corresponding to around 50% of the administered dose, was associated with the parent compound (Norris et al., 2001).

5.0 EFFECTS ON HUMANS

5.1 Acute exposure

In the USA and Australia, several different cyanobacterial toxins have been implicated in human illness from certain municipal water supplies, often after algal blooms had been treated with copper sulfate (Bourke et al., 1983; Falconer et al., 1983; Ressom et al., 1994). In most cases, the cyanobacteria and sometimes the toxins involved have been identified, but the levels of toxin associated with illness have not been established in any of the outbreaks. The Palm Island mystery disease, affecting about 140 people, largely children, in Australia, occurred after a dense cyanobacterial bloom on a water supply was treated with copper sulfate. Within a week, severe illness was seen, characterized by vomiting, hepatomegaly, and kidney dysfunction, with loss of electrolytes, glucose, and plasma protein; recovery took 1–3 weeks (Byth, 1980). The cause of illness was not initially identified although a subsequent cyanobacterial bloom in the same water supply reservoir was shown to be highly toxic (Hawkins et al., 1985). The causative organism was C. raciborskii, from which CYN was later isolated and characterized (Ohtani et al., 1992). An illness called Barcoo fever afflicting early settlers in Australia may also have been caused by consumption of C. raciborskii contaminated water (Hayman, 1992).

Effects following dermal exposure to CYN containing cyanobacterial cells were evaluated using skin-patch testing in humans (Pilotto et al., 2004; Stewart et al., 2006a). Exposed individuals showed mild irritation, but no statistically significant dose-response or time-response relationship was found between skin reactions and increasing cell concentrations for either whole or lysed cells (Pilotto et al., 2004). No detectable skin reactions were observed in individuals exposed to lyophilized C. raciborskii (Stewart et al., 2006b).

CYN was detected in water in 2 of 11 algal bloom-associated disease outbreaks described by the US Centers for Disease Control among users of freshwater lakes in 2009 and 2010: however, the co-occurrence of other cyanotoxins (MC and anatoxin-a) does not allow an estimation of the relative contribution to the multiple symptoms (nausea, vomiting, diarrhoea, abdominal cramps, anorexia) attributable to CYN (Hilborn et al., 2014).
5.2 Long-term exposure

There have been no studies of long-term human exposure to CYN.

6.0 EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO SYSTEMS

The majority of animal studies on CYN have used extracts of the producing cyanobacteria (usually *C. raciborskii* or *Aph. ovalisporum*), which is particularly problematic for understanding CYN toxicity because many studies have suggested that some effects in animals caused by extracts of *C. raciborskii* cannot be explained by the known CYN content of the extracts (Falconer *et al.*, 1999; de la Cruz *et al.*, 2013).

6.1 Acute exposure

Cattle deaths have been attributed to consumption of *C. raciborskii*-contaminated water (Thomas *et al.*, 1998).

A number of studies have investigated the acute oral toxicity of extracts of *C. raciborskii* (Falconer *et al.*, 1999; Shaw *et al.*, 2000, 2001; Falconer & Humpage, 2001) but no such studies have used purified CYN, with consequent limitations for the estimation of an LD$_{50}$. In male MF1 mice, the lowest lethal gavage dose of extracts of *C. raciborskii* strains PHAWT/M or PHAWT/1 was 4.4 mg/kg bw as CYN-equivalent, the highest non-lethal dose was 6.9 mg/kg bw, and the average lethal dose was approximately 6 mg/kg bw. Deaths occurred 2–6 days after treatment, and histopathology showed effects on the liver, spleen, thymus, heart, esophagus, gastric mucosa, and ocular orbits (Seawright *et al.*, 1999). When Quackenbush mice were given oral doses of *C. raciborskii* AWT 205 extract, mortality occurred in 2/4 mice at 6 mg CYN equivalent/kg bw (in 5 days) and 4/4 mice at 8 mg CYN equivalent/kg bw (in 24–48 hours) (Shaw *et al.*, 2000, 2001).

Single i.p. doses of purified toxin or toxic extracts induced a progressive toxicity over at least 7 days (Falconer *et al.*, 1999; Ohtani *et al.*, 1992; Terao *et al.*, 1994; Shaw *et al.*, 2000, 2001). Pathological lesions were observed in the liver, kidney, adrenal gland, lung, intestine, thymus and heart. The reported estimates of the i.p. LD$_{50}$ derive from the only study using purified CYN (Ohtani *et al.*, 1992) and depend on the observation time, being 2.1 mg/kg bw after 24 h or 0.2 mg/kg bw at 120–144 h or 5–6 days. The difference between the LD$_{50}$ values obtained following the oral and the i.p. administration are likely due to kinetic differences (Buratti *et al.*, 2017).

One acute toxicity study is also available after intratracheal injection of 70 μg/kg bw semipurified extracts to mice. Effects observed were impaired lung mechanics with parenchymal inflammation and production of inflammatory mediators, such as interleukins (IL-1b, IL-6) and neutrophil chemokines, induced alveolar collapse, polymorphonuclear cells, fiber deposition and oxidative stress (Oliveira *et al.*, 2012, 2015a,b).

Lyophilized extracts from CYN-producing cyanobacteria have been associated with moderate skin irritation and skin sensitization potential (Törökné *et al.*, 2001). However, cell components other than CYN could be the major causative agent since in the mouse ear swelling test purified CYN produced a response in only 22% of animals compared to an 80% response elicited by a *C. raciborskii* suspension (Stewart *et al.*, 2006c).
6.2 Short-term exposure

In mice given daily gavage doses of purified CYN for 14 days, the NOAEL (based on fatty infiltration of the liver) was 50 µg/kg and the LOAEL was 150 µg/kg (Shaw et al., 2001). Mice that were given 600 µg purified CYN/L in drinking-water for 3 weeks (estimated daily CYN dose of 66 µg/kg) showed increased liver and testes weights while urine excretion rate decreased. Liver cholesterol was reduced while cholesterol in the serum and erythrocyte membranes was increased (Reisner et al., 2004).

6.3 Long-term exposure

No chronic studies for CYN were identified. Subchronic exposure studies with CYN extracts have been conducted in mice via drinking-water exposure for 10 or 42 weeks or gavage exposure for 11 weeks (Humpage & Falconer, 2002, 2003). In male Swiss Albino mice given daily gavage doses of purified CYN for 11 weeks, the NOAEL was 30 µg/kg/d and the LOAEL was 60 µg/kg bw per day based on increased relative kidney weight. Other effects seen included decreased urinary protein and “minor” liver histopathology (≥ 120 µg/kg bw per day) and decreased urinary specific gravity, increased relative liver weight and renal proximal tubule damage (240 µg/kg bw per day) (Humpage & Falconer, 2002, 2003).

When *C. raciborski* AWT 205 extract was given to male Swiss Albino mice in drinking-water, CYN equivalent doses of 216, 432 or 657 µg/kg bw per day for 10 weeks caused reduced body weights (top two doses), increased relative liver and kidney weights, increased serum total bilirubin, decreased serum total bile acids (all at ≥ 216 µg/kg bw per day) and decreased urine protein concentration (g/mmol creatinine) at ≥ 432 µg/kg/d (Humpage & Falconer 2002, 2003).

Male and female ICR mice were given culture medium from toxic *Aph. ovalisporum* as their drinking-water for up to 42 weeks (Sukenik et al., 2006). The CYN concentration in the drinking-water increased from 100 to 550 µg/L over the dosing period so that the estimated daily CYN doses increased from 10 µg/kg to 55 µg/kg over the 42 weeks. Relative kidney weights were significantly increased in males and females at 20 weeks (when estimated daily dose was 30 µg/kg) and at 42 weeks. Relative liver weights were increased at 42 weeks, and relative testes weights were increased in males at 42 weeks. Perturbations of cholesterol distribution and erythrocyte morphology were also observed.

Recently, a detailed study of CYN toxicity in mice was reported by Chernoff *et al.* (2018). Male and female CD-1 mice were treated by gavage (0, 75, 150, 300 µg/kg bw per day in water) for 90 days. In this study there were clear gender-related differences seen in the pattern of toxic effects: although both sexes showed histopathological changes indicative of hepatotoxicity, females exhibited more marked adverse effects than males. Meanwhile, the reverse was true for renal effects: males exhibited significant renal histopathology at all doses tested while the females showed less marked effects and only at the two higher doses. Gene expression changes showed an up-regulation of the pro-apoptotic gene Bax and 60S ribosomal protein L6, which is involved in liver regeneration and expressed after liver injury. The fatty acid binding protein gene (Fabp4) that is associated with hepatic dysfunction was down-regulated. In the genes associated with coagulopathy, those produced in the liver and associated with inhibition of coagulation were significantly down-regulated whereas others involved in platelet function were not altered in this 90-day oral study, although they had been in previous i.p. studies (Chernoff *et al.*, 2014). No bleeding was seen in the 90-day study whereas it had been in the i.p. study, indicating that a relatively high effective dose may be required to produce
this effect. Significant effects were seen at 75 µg/kg bw per day in gene expression, liver and kidney body weight ratios and histopathology as well as blood urea nitrogen, so a NOAEL could not be identified.

In summary, available subchronic studies indicate that the liver and kidneys are major target organs for CYN-induced toxicity in mice; structural changes in erythrocytes and a haemorrhage/coagulopathy syndrome have also been observed. The lowest NOAEL for increased relative kidney weight was identified as 30 µg/kg bw per day. Decreased urinary protein excretion has also been reported following CYN administration, suggesting a specific effect on the nephron (Health Canada, 2016).

6.3.1 Reproductive and developmental toxicity

Sibaldo de Almeida et al. (Sibaldo de Almeida et al., 2013) did not find any visceral or skeletal malformations in the offspring of pregnant rats receiving oral doses up to 3 µg/kg/day purified CYN during Gestation Days (GD) 1-20.

In pregnant CD-1 mice receiving purified CYN via i.p. injections on GD 8–17, 3 daily doses of 64 µg/kg or greater were lethal to the majority of dams. Four of 20 dams given 5 daily doses of 32 µg/kg died. Relative liver but not kidney weight was significantly increased in surviving dams. No effects were seen on foetus weight, mortality, skeleton or soft tissues (Rogers et al., 2007).

In experiments in which pregnant CD-1 mice were given daily i.p. injections of 50 µg/kg bw per day, there was a much high mortality of dams treated during GD 8-12 (62%) than during GD 13-17 (3%). However, the surviving pups from the GD 13-17 group had reduced birth weight and postnatal growth compared to controls or pups from the GD 8-12 group (Rogers et al., 2007; Chernoff et al., 2011, 2014).

Human chorionic gonadotropin (hCG)-stimulated progesterone production was inhibited in primary cultures of human granulosa cells exposed to a non-cytotoxic concentration of CYN (1 µg/mL) for 24 h (Young et al., 2008). However, in a second experiment, CYN up to 3 µM (1.25 µg/mL for 6 h treatment) was not cytotoxic and did not alter production of progesterone or estrogen with or without hCG stimulation. Protein synthesis was significantly inhibited by 3 µM CYN alone and by 0.3 – 3.0 µM CYN when cells were stimulated with hCG (Young et al., 2012).

6.3.2 Genotoxicity and carcinogenicity

Bacterial mutagenicity studies (Ames Assay) have not shown mutagenic activity of CYN (Sieroslawska, 2013). However, various mammalian cell genotoxicity assays have shown positive effects. Human hepatocytic (HepaRG, HepG2), enterocytic (CaCo2) and lymphoblastoid (Wil2-NS) cell-lines have shown increased multinucleated and binucleated cells (Humpage et al., 2000; Bazin et al., 2010; Straser et al., 2011). DNA strand breaks were observed in primary mouse hepatocytes by the Comet assay (Humpage et al., 2005). In contrast, genotoxicity was not seen in Chinese Hamster Ovary cells (CHO-K1), possibly due to an inability of these cells to metabolically activate the toxin (Fessard & Bernard, 2003; Lankoff et al., 2007).

I.p. exposure caused DNA strand breakage in the liver of BALB/c mice (Shen et al., 2002) and
covalent binding between DNA and CYN, or a metabolite, in Quackenbush mouse liver (Shaw et al., 2000). Bazin et al., (2012) exposed male Swiss Albino mice to purified CYN either i.p. (50, 100, 200 µg/kg) or by gavage (1, 2, 4 mg/kg) and examined the occurrence of DNA damage by the Comet and micronucleus assays in various organs 24h after treatment. DNA damage was detected in the colon after i.p. injection of 100 and 200 µg/kg CYN, and in the colon and bone marrow after oral exposure to 4 mg/kg or 1 and 2 mg/kg, respectively.

Maire et al. (2010) investigated the in vitro carcinogenic potential of CYN using the cell transformation assay on Syrian hamster embryo cells. A significant ($p < 0.01$) increase in morphological cell transformation was seen at extremely low concentrations ranging from $1 \times 10^{-10}$ to $1 \times 10^{-5}$ μg/mL. Transformation was not seen at concentrations above $1 \times 10^{-5}$ μg/mL and cytotoxicity was not seen until 0.01 μg/mL. It was hypothesized that this pattern may have been caused by a CYN-induced decrease in cytochrome P450 enzymes at higher concentrations, as has been observed in vivo (Terao et al., 1994; Health Canada, 2016).

Swiss Albino mice were given up to 3 doses of extract of freeze-dried C. raciborskii AWT 205 over 6 weeks (2.75 or 8.25 mg CYN equivalent/kg bw/dose) and then fed liquid food with or without the tumour promotor tetradecanoylphorbol acetate (TPA) for 30 weeks. Neoplastic changes were found in five CYN-treated mice, but not in any of the 27 control mice, a difference that was not statistically significant. There was no pattern to the neoplastic changes, as they occurred in different animals, target organs and treatment groups, thus providing equivocal evidence for carcinogenicity (Falconer & Humpage, 2001).

6.3.3 Immunological effects

Although there have not been any specific studies into the potential immunotoxicity of CYN, a number of acute toxicity studies have reported necrotic lesions in the thymus and spleen following relatively high doses of extract or purified toxin (Terao et al., 1994; Seawright et al., 1999; Shaw et al., 2000, 2001).

In addition, in vitro studies reported that inhibition of lymphocyte proliferation in human peripheral blood samples was caused by 1 μg CYN/mL (Poniedziałek et al., 2012), possibly associated with oxidative stress induction and a reduced capacity to fight pathogenic microorganisms (Poniedziałek et al., 2014, 2015).

6.3.4 Hematological effects

Morphological changes to erythrocytes have been reported in some studies, an effect that may be linked to observed changes in cholesterol levels in the liver, plasma and erythrocyte cell membrane (Reisner et al., 2004; Sukenik et al., 2006). Many studies have reported hemorrhagic lesions in a range of tissues including the gastrointestinal tract, eye orbit, tail and vagina following exposure to C. raciborskii extracts or purified CYN (e.g., Shaw et al., 2000; Chernoff et al., 2011, 2014, 2018). The cause of these lesions has not been further investigated.

6.4 Mode of Action

Based on available studies, the liver, kidneys and erythrocytes may be important targets of CYN toxicity; however, the mode of action (MOA) for CYN-mediated toxicity is not fully elucidated. Although not clearly understood, the specific mechanism for liver toxicity may involve more than one mode of action (U.S. EPA 2015a) and depend on the magnitude and
frequency of dose, exposure duration, life stage, age or sex of the organism, and the duration that an animal is observed post-dosing (Buratti et al., 2017; Pichardo et al., 2017).

The MOA for liver toxicity could involve inhibition of protein synthesis (Terao et al., 1994; Froscio et al., 2003) that is not decreased by broad-spectrum CYP inhibitors, suggesting that it is mediated by the parent compound (Froscio et al., 2003). Potent inhibition of the isolated protein synthesis apparatus by purified CYN in vitro confirms this (Froscio et al., 2008).

Hepatocytotoxicity that occurs at higher CYN exposures appears to be CYP dependent, suggesting the involvement of metabolites and other mechanisms (Norris et al., 2002; Froscio et al., 2003; Humpage et al., 2005; Kittler et al., 2016). Pretreatment with the CYP inhibitor α-naphthoflavone partially protected against cytotoxicity and cellular GSH depletion (Runnegar et al., 1995): liver histopathology is mainly induced in the periacinar region where CYP-catalyzed xenobiotic metabolism occurs (Shaw et al., 2000, 2001); CYN-induced upregulation of genes coding for phase I enzymes (CYP1A1, CYP1B1, ALDH1A2 and CES2) and phase II enzymes (UGT1A6, UGT1A1, NAT1 and GSTM3) in HepG2 cells (Straser et al., 2013) are all in favour of metabolite-mediated toxicity, although in another study in mice all phase 1 enzymes examined with the exception of CYP2A4 were down-regulated (Chernoff et al., 2011) and no metabolites or reduction of CYN concentration were detected in vitro (Kittler et al., 2016). The specific enzymes involved have not been identified so far.

The role of ROS in CYN toxicity remains to be clearly defined. CYN can deplete mouse hepatic GSH in vivo (Norris et al., 2002) and decrease GSH levels and synthesis of GSH and protein in cultured rat hepatocytes (Runnegar et al., 1994, 1995, 2002). Inhibition of GSH synthesis was the predominant mechanism for the reduction in GSH; other mechanisms, including increased consumption of GSH, increased formation of oxidized glutathione, increased GSH efflux, hidden forms of GSH, decreased GSH precursor availability and decreased cellular adenosine triphosphate, were effectively ruled out (Runnegar et al., 1995). GSH depletion occurred at non-toxic CYN concentrations and preceded the onset of observable toxicity at higher concentrations (Runnegar et al., 1994). However, in CaCo2 cells, γ-glutamylcysteine synthetase (GCS) was induced and GSH levels were higher than control at higher CYN concentrations (Gutiérrez-Praena et al., 2012a). A toxicogenomic study on HepG2 showed that among the oxidative response genes only catalase (CAT) and thioredoxin reductase (TXNRD1) were upregulated (Straser et al., 2013). An in vitro study of CYN genotoxicity demonstrated DNA damage in the Comet assay without significant increases in ROS (Humpage et al., 2005).

In vitro evidence in cultured human dermal fibroblasts and HepG2 suggested that induction of stress responses may be involved in the MoA. Activation of the p53 transcription factor was observed along with concentration-dependent increases in mRNA levels of p53 target genes CDKN1A, GADD45a, BAX and MDM2 (Bain et al., 2007). Involvement of oxidative stress and ROS formation was confirmed by other studies: in human umbilical vein endothelial cell line (HUVEC) (Gutiérrez-Praena et al., 2012a); in HepG2 (0.05, 0.1 and 0.5 μg CYN/mL) (Straser et al., 2013); in primary rat hepatocytes (90–360 nM CYN) with induction of the transcription of the antioxidant response element (ARE)—binding factor, Nrf2 (Lopez-Alonso et al., 2013); in human intestinal Caco-2 cells (0.3 μg/mL) (Gutiérrez-Praena et al., 2012b).

Studies specifically investigating the inhibition of protein synthesis in the kidneys are not available, although the results of the 11-week oral toxicity study in mice (Humpage & Falconer, 2003) are consistent with an inhibition of protein synthesis.
OVERALL DATABASE AND QUALITY OF EVIDENCE

7.1 Summary of Health Effects

Human data on the toxicity of cylindrospermopsin are limited by the lack of quantitative information and by potential co-exposures to other cyanobacterial toxins and microorganisms (U.S. EPA, 2015a). No chronic exposure study in laboratory animals was identified. Available subchronic studies indicate that the liver and kidneys are major target organs for CYN-induced toxicity in mice; structural changes in erythrocytes have also been shown to be an endpoint of concern. The kidney appears to be the most sensitive target, with the lowest NOAEL for increased relative kidney weight identified as 30 μg/kg bw per day. Decreased urinary protein excretion has also been reported following CYN administration, suggesting a specific effect on the nephron.

The most appropriate study for derivation of guideline values for CYN is Humpage and Falconer (2003) in which the most sensitive effects observed were related to kidney damage (increased relative kidney weight at ≥ 60 μg/kg bw per day and decreased urinary protein at ≥ 120 μg/kg bw per day). This study identifies a NOAEL and LOAEL of 30 and 60 μg/kg bw per day, respectively. The findings of Humpage and Falconer (2003) have recently been corroborated by Chernoff et al. (2018) who identified a LOAEL of 75 μg/kg bw per day for changes in gene expression, liver and kidney to body weight ratios and histopathology as well as blood urea nitrogen.

7.2 Quality of Evidence

There are a number of major deficiencies in the toxicological database for CYN that limit confidence in the conclusions that can be drawn regarding its toxic potency and effects in humans. The majority of studies available have used Cylindrospermopsis extracts or poorly characterized toxin preparations and the intraperitoneal route of exposure. Other deficiencies include the lack of studies using highly pure and well characterized toxins, acute oral studies identifying a NOAEL, pharmacokinetic studies, oral studies corroborating effects on reproductive endpoints, chronic oral studies on toxicity and carcinogenicity, and oral studies on congener comparisons.

Nevertheless, some main themes have emerged, for example, the identification of protein synthesis inhibition as a key molecular initiating event mediated by the parent compound, and that metabolic activation appears to be important in mediating some other adverse outcomes including DNA and genomic damage in eukaryotic cells in vitro and in vivo.

8.0 PRACTICAL CONSIDERATIONS

Cylindrospermopsins are among the cyanotoxins found most frequently, although as discussed above, in most cases at quite low concentrations. Where blooms occur, concentrations of CYN can fluctuate due to uneven distribution of blooms in a water body, heterogeneity of clones within blooms and variation in the amount of toxin produced by individual clones.

Chapters 7 - 10 of TCiW give guidance on multiple barriers against cyanotoxins in water including controlling nutrient loads from the catchment, managing water bodies, optimizing sites for drinking-water offtakes or recreation, applying drinking-water treatment to remove
cyanobacteria and cyanotoxins and providing information or warnings for recreational use of water bodies with blooms. This includes guidance on planning, managing and documenting the barriers used to mitigate cyanotoxin risks through developing a water safety plan (TCiW; Chorus & McKeown, in press; Bartram et al., 2009).

8.1 Source control

For planktonic toxic cyanobacteria the prevention of blooms in source waters is the key to long-term control of the risks they represent. The most sustainable approach to achieve this is to keep concentrations of plant nutrients low. Most cyanobacteria typically proliferate under eutrophic conditions i.e., at elevated concentrations of nutrients, in particular of phosphorus, and total phosphorus concentrations below 20-50 µg/L will limit the development of cyanobacterial blooms in most situations (TCiW; Chorus & McKeown, in press; Zessner & Chorus, in press). A number of measures within water bodies can mitigate cyanotoxin occurrence, including e.g. artificial water column mixing, nutrient reduction through sediment removal or treatment, or biomanipulation. Their success is highly dependent on the specific conditions in the water body, as discussed in TCiW (Burch et al., in press).

Many reservoir off-take structures (towers) can take water from multiple depths to account for vertical heterogeneity. Variable off-takes enable avoiding water layers containing the highest concentrations of cyanobacteria. If multiple off-takes are not available (e.g. in small systems) it may be possible, as a temporary measure, to siphon water from a specific depth. However, this strategy may be less effective for CYN compared with other cyanotoxins due to the large extracellular fraction of toxin. Where conditions allow, the use of bank filtration between source waters and treatment plant inlets can be very effective both for removing cyanobacteria and for biodegradation of dissolved CYN (TCiW; Brookes et al., in press). Where possible, sites for recreational activities are best located upwind of bays where scums tend to accumulate.

8.2 Monitoring

Depending on a range of conditions, including climate, cyanobacteria can be present in surface waters throughout the year or as short-lived seasonal blooms, in both cases potentially producing significant concentrations of toxins. Monitoring of source waters should include surveillance for factors that can promote the growth of cyanobacteria including total phosphorus, temperature, water residence time, pH and Secchi disc transparency (for detail see TCiW; Padisák et al., in press). On site visual assessment of turbidity with greenish discoloration or scums and microscopy are effective low cost direct methods that can trigger increased vigilance if CYN-producing cyanobacteria are observed. In many cases monitoring over several seasons can establish the likely occurrence and timing of favourable conditions for cyanobacterial growth as well as the taxonomic composition and magnitude of blooms in individual water bodies. For example, a lake with regular seasonal blooms of *Aphanizomenon* in late summer is unlikely to shift to perennial blooms of *Cylindrospermopsis* from one year to the next (TCiW; Ibelings et al., in press).

Monitoring programmes should be adaptive with sampling and testing being increased when there is evidence of increasing cell numbers. Alert Level Frameworks (ALF) have been described both for drinking-water and for recreational water use. These include various criteria to trigger particular analyses and risk mitigation measures (TCiW; Humpage et al., in press; Chorus & Testai, in press;). As described in the ALFs, monitoring of source waters can start with simple site inspections for appearance of visible blooms, assessing transparency using a
Secchi disc. However, not all CYN producers form surface scums or strong discoloration, and these may be overlooked. Therefore, if the presence of cyanobacteria is suspected, microscopic examination for the presence of potentially CYN producing cyanobacteria is important. As blooms develop monitoring can be expanded to include quantitative measures of cyanobacterial biomass indicating potential toxin concentrations such as cyanobacterial biovolumes or chlorophyll-a, or direct analyses of CYN concentrations. However, while the detection of potentially CYN producing cyanobacteria indicates possible CYN occurrence, CYN dissolved in water may persist after the producing cyanobacteria have disappeared. Therefore, and also because concentrations associated with cyanobacterial blooms can vary substantially, where possible toxin analyses should be performed if CYN is suspected. Toxin data may well allow avoiding or lifting restrictions of site use where these were based on biovolume or chlorophyll-a concentrations.

8.3 Analytical methods and achievability

Analytical techniques are available for the range of parameters associated with cyanobacterial blooms and associated CYN. The complexity, expertise requirements and costs of monitoring increase from relatively simple visual inspections to testing for phosphorus, pH, Secchi disc transparency, cell numbers, species identification, biovolumes and chlorophyll-a determination. Testing for CYN using liquid-chromatography-mass spectrometry (LC/MS) or high-performance liquid mass spectrometry (HPLC) is the most complex and time consuming.

For cell-bound CYN an extraction step is performed prior to analysis. Due to the high hydrophilicity of CYN, polar extraction solvents such as water or methanol/water have been found to be effective. For dissolved CYN, sample concentration may be required to reach detection limits below guideline values and can be achieved with solid phase extraction using polygraphite carbon or polymeric sorbent.

A variety of analytical methods for CYN are available. The most commonly used methods to analyze CYN apply HPLC or LC-MS. USEPA Method 545 based on Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS) has a detection limit well below 0.7 µg/L. Certified reference material for CYN analysis is commercially available, and commercial immunoassays are available. Commercially available ELISA kits report a quantification range between 0.05 - 2 µg/L (for more details, see TCiW; Metcalf et al., in press).

While these methods were developed for the analysis of water samples, applying them to more complex matrices such as food or stomach contents requires prior clean-up.

Molecular methods have been developed to identify the presence of genes involved in the production of CYN. These methods do not provide information about actual toxin production or concentrations but can provide early warning of potential toxicity.

8.4 Treatment methods and performance

Treatment processes to reduce CYN in drinking water are based on two approaches; reducing cell-bound CYN by physical removal of the cells and reducing dissolved CYN (TCiW; Newcombe et al., in press). Unlike other cyanotoxins a high proportion of CYN can be dissolved in the water column. Nonetheless, a relevant proportion of CYN is likely to be cell-bound and therefore effectively removable by physical processes, i.e. coagulation followed by flocculation, clarification and rapid media filtration as well as by slow sand filtration or
membrane filtration. Care needs to be taken to avoid or minimise pre-filtration treatments such as chlorination as this causes cell lysis and release of CYN. Further, as cells may lyse in more acidic water, the pH should be kept above 6. Care also needs to be taken to ensure that cyanobacterial and CYN concentrates (e.g. filter backwash, sludges and sludge supernatants) are not allowed to return to the head of the filtration plant during a bloom. Other physical removal processes such as slow sand filtration, conventional flocculation with sedimentation and filtration or dissolved air flotation with filtration, and membrane filtration can also be very effective in removing cell bound CYN.

Dissolved CYN can be removed by adsorption onto powdered or granular activated carbon (PAC or GAC). Efficacy of removal can be influenced by the type of activated carbon, doses and points of application (PAC), contact times (PAC), flow rates (GAC) and water quality. Biological degradation of CYN during slow sand filtration and on GAC filters can be very effective, although it may require a lag phase for the degrading bacteria to establish.

Oxidation by chlorine or ozone can be effective for destruction of dissolved CYN under conditions normally applied for optimal disinfection of drinking-water. However, other oxidants such as chloramine and chlorine dioxide have been shown to be ineffective against CYN. Ozone or chlorine can be used to reduce concentrations of CYN prior to filtration. Also, where removal of cells by filtration is inadequate or absent, if applied in sufficient amounts these oxidants can both lyse the cells and destroy the released toxins. Moreover, elevated cyanotoxin concentrations typically occur during blooms which cause a high organic load to the treatment plant. As oxidising this without prior filtration is likely to cause high concentrations of disinfection byproducts, filtration prior to oxidation is recommended.

The treatment methods discussed above are able to reduce CYN concentrations below 0.7 μg/L. However, validation of efficacy under specific local conditions is important, this applies in particular to slow sand filtration and chlorination: their efficacy is highly dependent on the specific water quality and further conditions in the treatment system. Validation may include field trials and laboratory investigations such as jar testing. Verification of removal during blooms should be undertaken by monitoring of CYN in the finished drinking-water.

After effective treatment it is important to ensure drinking-water remains safe and free of cyanobacterial regrowth. This can be accomplished by ensuring that any channels and storages are covered and dark, so that cyanobacteria lack light necessary for growth. Maintaining chlorine residuals throughout the distribution system will also suppress cyanobacterial regrowth.

9.0 CONCLUSIONS

9.1 Derivation of the provisional guideline-values

The Point of Departure (PoD) has been identified as the NOAEL of 30 μg/kg bw per day from the Humpage & Falconer (2003) study. By applying an uncertainty factor (UF) of 1000 (100 for inter and intraspecies variability and 10 for the lack of chronic toxicity studies and deficiencies in the toxicological database), a provisional TDI (NOAEL/UF) value of 0.03 μg/kg bw per day can be derived. The value is provisional due to deficiencies in the CYN toxicological database, essentially related to the limited availability of studies with purified
toxins, the unclear role of metabolites and evidence of genotoxicity. The Sukenik et al. (2006) 42-week drinking-water study is considered supporting qualitative evidence for CYN toxicity, but the experimental design does not allow derivation of a robust reference value (Funari & Testai, 2008). The study by Chernoff et al. (2018) observed many of the same effects as seen previously and demonstrates that the NOAEL is below 75 μg/kg bw per day.

The toxicological database is more limited for CYN compared to microcystin-LR, and critically, there is indication for in vivo genotoxicity by CYN. Therefore, an uncertainty factor of 3 was used to allow for these uncertainties in the derivation of the provisional short-term and recreational guideline values.

Calculation of provisional lifetime drinking-water guideline value for cylindrospermopsin:

\[
GV_{CYN,chronic} = \frac{NOAEL \times bw \times AF}{UF \times C} = \frac{30 \times 60 \times 0.8}{1000 \times 2} \mu g L^{-1} = 0.72 \mu g L^{-1} \approx 0.7 \mu g L^{-1}
\]

AF: proportion of exposure assumed to be due to drinking-water, assumed to be 0.8 for drinking-water, as other sources of exposure such as air, food, and soil are considered minor;
bw: adult body weight (WHO standard = 60 kg);
C: daily water consumption, assumed to be 2 L for an adult;
GV_{chronic}: guideline value for chronic (lifetime) exposure;
NOAEL: no observed adverse effect level (30 μg/kg per day, based on Humpage and Falconer (Humpage & Falconer, 2003);
UF: uncertainty factor (1000: 100 for interspecies and intraspecies extrapolation x 10 for database deficiencies, including lack of a chronic study).

Calculation of provisional short-term drinking-water guideline value for cylindrospermopsin:

To develop a short-term guideline value, the same logic was applied except that a UF of 3 was used for database limitations:

\[
GV_{CYN,shortterm} = \frac{NOAEL_{subchronic} \times bw \times AF}{UF \times C} = \frac{30 \times 60 \times 1.0}{300 \times 2} \mu g/L = 3.0 \mu g/L
\]

AF: proportion of exposure assumed to be due to drinking-water, assumed to be 1.0;
bw: body weight (WHO standard = 60 kg);
C: daily water consumption, assumed to be 2 L for an adult;
GV_{shortterm}: guideline value for short-term exposure;
NOAEL: no-observed-adverse-affect level (30 μg/kg bw per day, based on Humpage and Falconer;
UF: uncertainty factor (300: 100 for interspecies and intraspecies extrapolation x 3 for database deficiencies).

Calculation of provisional recreational water guideline value for cylindrospermopsin:

\[
GV_{CYN,recreation} = \frac{NOAEL \times bw}{UF \times WI} = \frac{30 \times 15}{300 \times 0.25} \mu g/L = 6 \mu g/L
\]
bw: body weight (15 kg for a child); 
WI: incidental water intake (250 mL during primary contact- e.g. energetic play, swimming, falling out of a boat) 
GV_recreation: guideline value for recreational exposure; 
NOAEL: no-observed-adverse-effect level (30 µg/kg per day, based on Humpage and Falconer, 2003); 
UF: uncertainty factor (300: 100 for interspecies and intraspecies extrapolation x 3 for database deficiencies).

9.1 Considerations in applying the provisional guideline values

Informing the public about cyanobacterial blooms in source waters is important particularly if toxin concentrations in finished drinking-water temporarily exceed the lifetime guideline value. Furthermore, cyanobacterial blooms tend to impair the taste and odour of drinking-water, and informing the public about the safety of its use is important in order to avoid people turning to other, less safe sources of water.

For recreational sites with blooms, information and warnings are particularly important. The most common situation is that monitoring cannot occur at sufficiently short time intervals (i.e. daily rather than weekly) to ensure that it captures situations with heavy scums. Site users therefore need information about avoiding scum contact and ingestion as well as situations with pronounced greenish turbidity, i.e. to the extent that one cannot see one’s feet when knee-deep into the water. Temporary closure of sites is an option if blooms contain high toxin concentrations, exceeding the recreational guideline values (for further detail see TCiW, d’Anglada et al.).

The provisional guideline values are based on toxicological data for CYN. The limited evidence on the relative potency of other CYN congeners suggest they are probably similar to that of CYN. Therefore, it is suggested that these compounds be treated as equivalent to CYN (on a molar basis) for risk management purposes.

The provisional lifetime guideline value applied an allocation factor of 80% because drinking-water is usually the most likely long-term source of exposure. However, in some regions, food may be a significant source of exposure, particularly in tropical locations where the duration of blooms is long and there is high consumption of local seafood. In such situations, consideration should be given to reducing the allocation factor based on relative consumption data from the exposed population.

Closure of a water supply can have a range of adverse consequences. Many cyanobacterial blooms are episodic and short-lived. The short-term drinking-water guideline value is intended to provide guidance on how much the lifetime guideline value can be exceeded for short periods of about 2 weeks until enhanced water treatment can be implemented. It is not intended to allow for repeated seasonal exceedances of the lifetime guideline value.

The short-term drinking-water guideline value is based on exposure of adults. Since infants and children can ingest a significantly larger volume of water per body weight (e.g. up to 5 times more drinking-water/kg bw for bottle-fed infants compared to an adult), it is recommended that alternative water sources such as bottled water are provided for bottle-fed infants and small children when CYN concentrations are greater than 0.7 µg/L even for short periods, as a precautionary measure.
Unlike the other cyanotoxins, most CYN is found outside the producing cells within the water column. *C. raciborskii* does not provide warning of its presence by forming surface scums. Some studies have shown a poor correlation between cell number of the assumed producing organisms and the toxin concentration. These considerations necessitate a different risk assessment and risk management approach for CYN. For example, ELISA may provide more accurate data than cell counts as the primary monitoring tool. During water treatment, activated carbon may be necessary to remove dissolved toxin rather than relying on cell removal by sand filtration.

### 10.0 REFERENCES (still need some tidying up)


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