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Health Risk Assessment of Cyanobacterial (Blue-green Algal) Toxins in Drinking Water

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Abstract: Cyanobacterial toxins have caused human poisoning in the Americas, Europe and Australia. There is accumulating evidence that they are present in treated drinking water supplies when cyanobacterial blooms occur in source waters. With increased population pressure and depleted groundwater reserves, surface water is becoming more used as a raw water source, both from rivers and lakes/reservoirs. Additional nutrients in water which arise from sewage discharge, agricultural run-off or storm water result in overabundance of cyanobacteria, described as a 'water bloom'. The majority of cyanobacterial water-blooms are of toxic species, producing a diversity of toxins. The most important toxins presenting a risk to the human population are the neurotoxic alkaloids (anatoxins and paralytic shellfish poisons), the cyclic peptide hepatotoxins (microcystins) and the cytotoxic alkaloids (cylindrospermopsins). At the present time the only cyanobacteral toxin family that have been internationally assessed for health risk by the WHO are the microcystins, which cause acute liver injury and are active tumour promoters. Based on sub-chronic studies in rodents and pigs, a provisional Guideline Level for drinking water of 1μg/L of microcystin-LR has been determined. This has been adopted in legislation in countries in Europe, South America and Australasia. This may be revised in the light of future teratogenicity, reproductive toxicity and carcinogenicity studies. The other cyanobacterial toxin which has been proposed for detailed health risk assessment is cylindrospermopsin, a cytotoxic compound which has marked genotoxicity, probable mutagenicity, and is a potential carcinogen. This toxin has caused human poisoning from drinking water, and occurs in water supplies in the USA, Europe, Asia, Australia and South America. An initial health risk assessment is presented with a proposed drinking water Guideline Level of 1μg/L. There is a need for both increased monitoring data for toxins in drinking water and epidemiological studies on adverse health effects in exposed populations to clarify the extent of the health risk.

Keywords: health risk, cyanobacteria, toxins, drinking water

Introduction

Cyanobacterial toxins are well recognized as a cause of livestock poisoning, which has been extensively reported in the Americas, Europe, Asia and Australasia [1]. Livestock are inevitably vulnerable to poisoning as they are restricted in access to water by topography and by fences, and hence may have no choice but to drink water infested by toxic cyanobacteria. Most stock deaths have resulted from the formation of cyanobacterial water-blooms in ponds and lakes on farms, but several major poisoning events were through water blooms on rivers and drinking water reservoirs [2, 3].

Human poisoning has also occurred, but the reports are less well documented. The symptoms of poisoning by the main toxic cyanobacteria in drinking water reservoirs overlap with a range of other gastrointestinal illnesses, largely caused by infectious disease organisms. As a consequence during an outbreak of enteric disease the pathogens are investigated first, as the most probable cause, and only after exhaustive exploration are toxins of any type evaluated. Agricultural chemicals and industrial pollutants such as heavy metals are likely to be next suspected, with cyanobacterial toxins ignored until well after the event [4].

Epidemiological data for human poisoning by cyanobacterial toxins only exists for a small number of events. The most well characterized case was the poisoning of renal dialysis patients in a clinic in Caruaru, Brazil, in 1996. In this instance the patients treated in a dialysis clinic during one week suffered severe illness following perfusion, with hepatic failure and, in more

than 50 cases, death. Investigation of the water treatment unit at the clinic found contamination of the filters by two types of cyanobacterial toxin, microcystins and cylindrospermopsins [5, 6]. Microcystins were detected in the blood and liver of poisoned individuals [7]. Because of the severity of the poisoning a thorough investigation was carried out, which showed up major defects in the operation of the water treatment unit at the clinic. Exposure to toxins through renal dialysis is a particularly potent route of poisoning, equivalent to an intravenous injection in the case of water soluble toxins. The volume of water used in perfusion is large, about 120L, so that the total amount of toxin to which a dialysis patient is exposed is much greater than possible through drinking water.

Exposure to cyanobacterial toxins through consumption of contaminated drinking water has however also resulted in poisoning. The earliest demonstration of this was in 1983, when the population of a rural town in Australia was supplied with drinking water from a reservoir carrying a dense water bloom of a toxic species of cyanobacterium, *Microcystis aeruginosa.* The toxicity of this water bloom was being monitored in the reservoir. The controlling authority dosed the reservoir with copper sulphate to destroy the cyanobacteria, which caused the cells to lyse and release toxin into the water. Epidemiological data for liver injury in the affected population, a control population and comparison of the time periods before the bloom, during the bloom and lysis, and afterwards, showed clearly that liver damage had occurred only in the exposed population and only at the time of the water bloom [8]. In another less well characterized event, about 140 children and 10 adults were hospitalized, after the water supply authorities treated a cyanobacterial bloom in a small drinking water supply reservoir with copper sulphate, to resolve taste and odour problems. Within a week severe hepatoenteritis was apparent in the population, with about 20 cases requiring intravenous therapy. No-one died though several children were placed in intensive care [9]. Subsequent investigation demonstrated a "new" toxic cyanobacterial species in the reservoir, with a potent general toxin [10]. Later work on this strain of cyanobacterium lead to the identification of an alkaloid cytotoxin, with considerable liver toxicity [11].

How Abundant are Cyanobacterial Toxins?

Cyanobacteria are a normal component of the worldwide biota, with a wide tolerance of climatic conditions and environment. As a very ancient life-form they occupy every conceivable ecological niche, and their abundance is limited by nutrient and light availability [12]. In aquatic systems cyanobacteria are always present, through the population density varies from very small numbers to more than 10^6organisms/mL. There is a strong relationship between phosphorus concentration in the water and cyanobacterial numbers and also a similar though less linked relationship between dissolved nitrate/ammonia and cyanobacteria [13]. Thus in general toxic cyanobacterial species will be present in all water bodies, with numbers dependent on the available nutrients and light.

As human population density rises the inflow of nutrients into water bodies increases through agricultural fertilizer use, urban run-off and sewage discharge. This increase in aquatic nutrients is termed eutrophication, and it is observed worldwide. Phytoplankton in general becomes more abundant, and among these organisms are the cyanobacteria. Cyanobacteria can utilize nutrients competitively with eukaryotic phytoplankton, and will proliferate more successfully at lower nutrient concentrations than the green algae. As a result many rivers, lakes and reservoirs worldwide develop high cyanobacterial cell concentrations, especially in the summer months, which appear as greenish suspensions in the water. Some species float to the surface under warm, still conditions, forming scums with extreme cell concentrations above $1x10^6$cells/ml. Dried scums often appear blue-green or red through liberation of phycocyanin pigment, leading to the common name of these organisms - blue-green algae.

The scum-forming cyanobacterial species are largely toxic, and the majority of domestic animal poisonings have occurred from the animals drinking scum [12]. Cell populations carry over from year to year, and once a reservoir or lake has an established water bloom of cyanobacteria in summer, it is very difficult to reverse this phenomenon. Cyanobacteria proliferate in warmer weather, and often form extensive blooms in late summer. With an increase in global temperatures, cyanobacterial populations are likely to increase also. With the growth in human populations, demand for drinking water has resulted in water is being drawn from water bodies carrying substantial cyanobacterial populations, thus presenting a risk to human populations.

Annual or even permanent blooms of toxic cyanobacteria are becoming increasingly common in drinking water reservoirs. To give an illustration the three main reservoirs supplying Brisbane in Australia all carry substantial populations of the toxic *Cylindrospermopsis raciborskii.* This cyanobacterium forms dense layers 5-10m below the surface, so that the first indication of the proliferation of the organism may be the blocking of filters in the drinking water treatment plant. Other examples are the main drinking water supply reservoirs for the cities of Sao Paulo in Brasil and Lodz in Poland, which contain heavy blooms of the toxic *Microcystis aeruginosa in* summer.

Cyanobacterial Toxins

Neurotoxins form one of the major groups of cyanobacterial toxins. They are produced by several genera of cyanobacteria growing in freshwater which have the capacity to form dense waterblooms and floating scums at the edge of lakes and rivers. The neurotoxins are alkaloid compounds, fast acting and have caused many deaths of dogs and livestock [1]. Three types of alkaloid have so far been described (Figure 1).

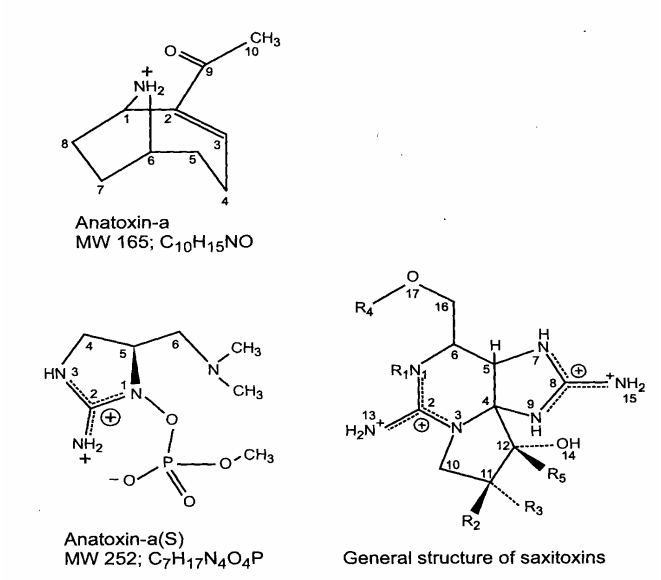

Figure 1: Structures of cyanobacterial neurotoxic alkaloids.

The first to be characterized was anatoxin-a (Fig.1), a neuromuscular blocking agent which causes death by respiratory paralysis [14]. This toxin has been found in three common genera of cyanobacteria, *Anabaena*, *Aphanizomenon* and *Planktothrix*, all filamentous planktonic organisms capable of high cell concentrations and potential scum formation. There has been no clear evidence of human poisoning from these organisms, though a coroner in Wisconsin in 2003 resolved that the death of a male teenager who was diving and playing in a pond containing neurotoxic *Anabaena* had died as a consequence of ingestion of these cyanobacteria [15] To verify this cause of death, evidence of toxin in the gastrointestinal tract or tissues was required, however there has been no published report of the presence of toxin. By contrast, dogs poisoned by anatoxin-a have shown the toxin in stomach contents [16]. The compound is stable in the environment, as exhibited by the dogs having died after eating decaying lumps of cyanobacteria on the lakeside. Anatoxin-a has been identified in the water of lakes in North America and in Europe, which are largely used for recreation. There is the possibility of consumption of moderate quantities of water during swimming and especially water skiing, and hence a risk exists for anatoxin-a poisoning of recreational water users [17]. Many authorities in developed countries have warning procedures for cyanobacterial blooms at popular recreational areas, to reduce risk to water users [18]. There has been little attention paid to the assessment of risk to drinking water consumers from anatoxin-a, largely because of the rapid excretion of the toxin from the body, no evidence of residual effects and low free-water concentrations in lakes.

Anatoxin-a(s) is much less common in cyanobacterial waterblooms, though it was first identified following cattle deaths in the USA[19]. The alkaloid closely resembles an organophosphorus insecticide (Figure 1), and acts as an anticholinesterase. The characteristic feature of this poisoning is excessive salivation, which is the reason for the designation (s). The compound is highly unstable and unlikely to persist in water supplies, and as a result is also unlikely to present any risk.

The saxitoxin-type neurotoxins are well known as the cause of paralytic shellfish poisonings, which have resulted in many hundreds of human deaths worldwide [20]. As a result, legislation controls the allowable concentration of saxitoxins in shellfish harvested for human consumption (80μg/100g fresh shellfish tissue) and there is a substantial monitoring program in many countries. Saxitoxins are however not limited to marine waters, and also occur in freshwater cyanobacteria. *Anabaena, Aphanizomenon* and *Lyngby*a genera of cyanobacteria have species that produce saxitoxins [21-23]. The massive waterbloom of *Anabaena circinalis* on 1,000km of the Darling River in Australia in 1991 killed a large number of sheep and cattle, and also resulted in detectable neurotoxicity in town water supplies [24]. Saxitoxins are heat-stable molecules, which are not easily removed in conventional water treatments unless pH and chlorine residuals are carefully controlled, but can be effectively removed by ozone or activated carbon [25].

The toxicity of saxitoxin is considerable, as the alkaloid blocks sodium conduction in axons preventing nerve impulse transmission, leading to paralysis. The oral LD_{50} in mice is about 260μg/kg bodyweight [26]. Acute poisoning in humans is unlikely to occur from contaminated water supplies, as the human body can tolerate about 100μg of saxitoxin without ill effect [20], which translated to drinking water is 50μg/L assuming 2L water drunk per day. No cumulative effects have been demonstrated, though there is limited evidence of resistance to toxicity in exposed human populations [26]. New Zealand is considering a Maximum Acceptable Value in drinking water of 3μg/L of saxitoxin equivalents in their new drinking water guidelines, which should be ratified shortly [27]. This value has also been proposed in Australia [28]. The data on which this value was based were the intraperitoneal toxicity of saxitoxin to mice, and incorporated a safety factor of 1,000. This issue will be discussed further in the section on hepatotoxins (microcystins). There are no data for the concentrations of saxitoxin-type neurotoxins in drinking water, and there were no reports of neurotoxic symptoms in the town population when neurotoxicity was detected in the drinking water supply [29].

Hepatotoxins

These toxins have received the greatest attention, as they are the source of the most likely risk to consumers of drinking water. The predominant genera of cyanobacteria forming the peptide toxins called microcystins are *Microcystis, Planktothrix* and *Anabaena.* Species from these genera are common in Europe, the Americas, Africa and Asia, and poisoning of domestic animals has been widely reported [12]. Only two epidemiological investigations have so far shown human injury from microcystin in drinking water, one in Australia [8] and one in China (unpublished). As a consequence of the frequency of cyanobacterial blooms containing hepatotoxins in drinking water reservoirs, the WHO carefully examined the need for the major toxins, the

microcystins, to be included in the drinking water guidelines. An 'expert group' was established to examine the whole issue of cyanobacterial toxins in drinking water, which resulted in a comprehensive assessment of the risks involved [30]. The outcome was a recommendation that the microcystins should be included among the chemicals for which Guideline Values be determined. These peptide toxins are cyclic, and contain a majority of D-amino acids (Figure 2). The positions shown as [X] and [Y] are L-amino acids, and are variable between species and strains of cyanobacteria. The most abundant variant has L-leucine (L) and L-arginine (R) respectively at [X] and [Y] (microcystin-LR). The amino acid at the left of the molecule is unique, is connected into the ring through an amino group at the β-carbon atom, and has the trivial name of ADDA.

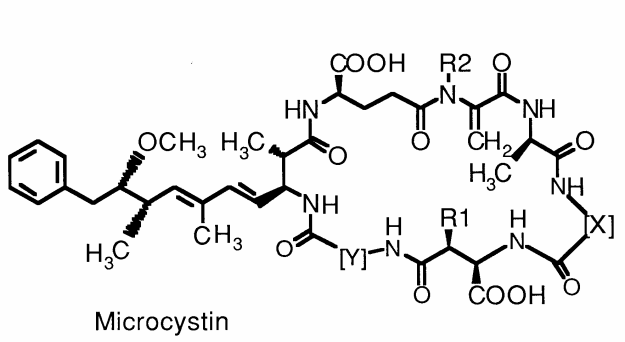

Figure 2: Structure of the peptide hepatotoxin microcystin, first isolated from the cyanobacterium *Microcystis aeruginosa.*

Microcystins are resistant to digestion in the gastrointestinal tract of eukaryotes, as peptide bonds linking to the D-amino acids are not susceptible to normal hydrolytic enzymes. The toxins are concentrated into the liver by an active transport system, similar to the bile acid transporter [31]. Microcystins specifically inhibit protein phosphatases 1 and 2A, which have a vital role in cell control and in intracellular structure [12]. Acute poisoning is through destruction of the liver architecture, leading to blood loss into the liver and hemorrhagic shock [32]. Later death is through liver failure with massive destruction of hepatocytes, seen in large animal deaths and human fatalities [5, 33]. Chronic exposure to these toxins in drinking water led to ongoing active liver injury in mice [34].

There is experimental evidence for tumour promotion by microcystins, and limited data for carcinogenesis in rodents [35]. In rural areas in Southern China some villages showed hyper-endemic rates of hepatocellular carcinoma, which have been shown to be linked to hepatitis, aflatoxin in the food, and drinking surface water. Microcystins in the ponds and ditches used as water sources were suspected of contributing to the cancer rates [36, 37].

These toxins are highly stable in water and are resistant to boiling. Hence they present a risk to consumers in less developed regions and countries who are collecting water from surface sources to drink. Many lakes, ponds, ditches and streams in rural and outer urban areas suffer from eutrophication through excessive nutrient leaching from housing, sewage, and intensive agricultural use, leading to cyanobacterial proliferation. In tropical and temperate regions of the world the genus *Microcystis* is the most abundant cyanobacterium forming toxic blooms, with toxin concentrations sufficient to poison domestic animals. If these contaminated water sources are used for human consumption, there is a risk of human poisoning. Conventional Western drinking water treatment may not be effective under bloom conditions in removing microcystins from drinking water and hence there is a risk to consumers. Advanced water treatment using ozone and activated carbon will reliably remove microcystins [12].

WHO have carried out an assessment of the safe level of microcystins in drinking water, based on data from a subchronic toxicity trial in mice, with supporting data from growing pigs [38]. The calculation used the No Observed Adverse Effect Level for male mice during a 13 week oral toxicity trial, of 40μg of microcystin-LR/Kg/day [39]. This was used to calculate a Tolerable Daily Intake (TDI) for safe human consumption, by the incorporation of uncertainty or safety factors. While these are subjective, a factor of 10 for interspecies uncertainty between rodents and humans, a further 10 for variability in sensitivity between people, and an uncertainty of 10 for inadequate data, possible tumour promotion and lack of lifetime exposure are generally accepted.

Thus the:

$$TDI = \frac{40}{10 \times 10 \times 10} = 0.04\mu g/Kg/day$$

From this value the Guideline Value (also called the reference dose and the maximum acceptable concentration) was calculated from the standard bodyweight of 60Kg, an assumption of the proportion of the dose from drinking water of 0.8 (some may come from food and particularly blue-green algal diet supplements) and a standard water consumption of 2L/day.

$$GV = \frac{0.04 \times 60 \times 0.8}{2} = 0.96$$

$= 1\mu g/L$ of microcystin-LR in drinking water.

This value was determined from the toxicity of microcystin-LR, so the WHO Chemical Safety Committee set the Guideline Value for microcystin–LR. Since there are some 60 variants of the molecule, and some highly toxic blooms do not contain any of the –LR variant, it is necessary to interpret this as toxicity equivalent to microcystin-LR. Where this Guideline Value has been adopted as the basis for national legislation, the need for monitoring of all the toxin variants has been recognized and the equivalent total toxicity calculated [40] . Individual countries have also adopted a higher standard bodyweight, and a different proportion of the consumption from drinking water. All of the Guideline Values adopted so far lie between 1 and

2μg/L of microcystin equivalents, which for practical purposes are the same.

A larger potential adjustment to this value may result from re-classification of microcystin as a carcinogen, rather than a non-carcinogenic poison. While there is experimental evidence for tumour promotion by microcystin in liver, skin and colon, the only data indicating carcinogenesis have been obtained by continued very high intraperitoneal doses of toxin in mice which cause extensive liver damage [1]. In China there is ongoing investigation into the relationship between surface water consumption and cancer of liver and colon [41]. This issue is discussed in detail elsewhere [12], concluding that there is insufficient evidence at present to determine that microcystin is a probable carcinogen but the possibility requires continual evaluation.

Cylindrospermopsins

These alkaloid cytotoxins were relatively recently discovered, following the widespread human poisoning at Palm Island, Australia due to contamination of the water supply [9]. Cyanobacteria from the supply reservoir were collected, cultured and evaluated for toxicity, showing potent toxicity to liver, kidney, adrenals, lymphoid cells and other tissues in mice [10]. Subsequent investigation of the oral toxicity of the cyanobacterium responsible, *Cylindrospermopsis raciborskii*, further demonstrated the tissue damage caused by the toxin [42, 43]. The toxic alkaloid was isolated and identified as a potent inhibitor of protein synthesis [11, 44]. There are on-going investigations into the mechanism of cylindrospermopsin toxicity, which may involve activated metabolites of the alkaloid [44, 45]. The alkaloid has several reactive groups, including a hydroxymethyl uracil, which may be vulnerable to biological oxidation (Figure 3).

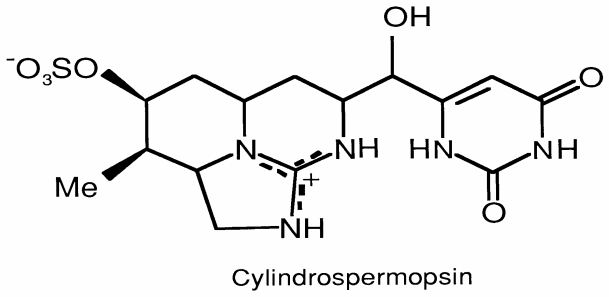

Figure 3: Molecular structure of the cyanobacterial alkaloid toxin cylindrospermopsin. The bridging hydroxyl group may be in either stereochemical position, and may also be replaced by hydrogen.

Cylindrospermopsin has been found in water bodies that have blooms of the cyanobacterial species *Aphanizomenon ovalisporum* [46], and *Umezakia natans* [47] as well as those with *Cylindrospermopsis* [48, 49], and recently in Germany, in the absence of any of those species [50]. It is apparent from this data that cylindrospermopsin is likely to occur widely in freshwater sources, and that only a beginning has been made in identifying species producing this toxin. Monitoring of water supplies for cylindrospermopsin has found concentrations in natural water bodies, reservoirs and in drinking water that are above 10μg/L, which is a cause for concern [51, 52].

On the basis of the experimental toxicity of cylindrospermopsin, the reported human poisoning associated with *Cylindrospermopsis* and the toxin concentrations measured in water bodies, it was apparent that risk assessment for this toxin in drinking water was required. A subchronic oral exposure trial of cylindrospermopsin in male mice provided a No Observed Adverse Effect Level of 30μg of cylindrospermopsin/Kg/Day. On the assumption of standard uncertainty factors and the total intake arising from drinking water, a Guideline Value of 1μg/L resulted [53].

Examination of the molecular structure of cylindrospermpsin indicated that it may be able to interact with DNA or RNA in cells, through the uracil group, assisted by the planar shape of the molecule. If this proves to be the case, then evaluation of the possible carcinogenicity of the molecule is required. Preliminary data indicated that cylindrospermopsin may form DNA adducts [54], and there is evidence for clastogenicity and micronucleus formation in a cultured human white cell line incubated with cylindrospermopsin [55]. A preliminary trial of carcinogenicity in mice indicated the presence of excess tumors, providing support for a more definitive carcinogenicity trial [56]. It is premature at present to attempt to classify cylindrospermopsin as a possible human carcinogen, because of the very limited current data. Further experimental and epidemiological research on this toxin is required to clarify these issues, and cylindrospermopsin is now on the 'Candidate Contaminant List' of the US Environmental Protection Agency.

Evaluation of the Risks to the Population from Cyanobacterial Toxins.

There are two areas in which more data is necessary to make a clear case for national action on minimizing health risks from cyanobacterial toxins. The first is the need for widespread monitoring for the presence of toxic cyanobacterial species and toxins in drinking water sources, to identify the abundance of locations of potential risk. This is in progress in Europe, and an initial survey has been carried out through the American Water Works Association in the USA. From data arising from these surveys, the extent of the problem has become apparent, and the location of a proportion of the populations at most risk.

The second and more difficult aspect is the need for epidemiological studies on at-risk populations to quantify the adverse health effects. Exposure biomarkers will have to be developed, in addition to quantitating the toxin concentrations in tap water. The commonly used clinical measures of liver and kidney function, and clinical records for hepatoenteritis, provide relevant health information. The earlier data on population injury

from microcystins indicates the clinical parameters of particular interest [8].

Conclusion

The cyanobacterial toxins provide a risk to human health when the population of toxic cyanobacteria in drinking water sources rises to bloom proportions. The present assessments of Guideline Values for these toxins as chemical, non-carcinogenic contaminants indicate that a safe concentration in drinking water is in the region of 1μg/L, a concentration that has been exceeded in numerous water storages. Carcinogenicity of these toxins is not yet established, though both microcystins and cylindrospermopsin have caused excess tumors in rodent experiments. With the increased eutrophication of water supplies and global warming, cyanobacterial populations and hence toxic risks are likely to rise in the immediate future. The extent and potential severity of the risks need further evaluation.

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