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Cyanobacterial Toxins, Drinking Water and Human Health

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Abstract : The occurrence of toxic cyanobacterial blooms has been reported worldwide and poses a threat to human health through drinking water exposure. The toxins they produce are highly water soluble and can leach into the water body. To eliminate any risk of drinking water exposure, removal of these toxins is essential before the water is consumed. Conventional water treatment techniques such as chlorination, if managed well, can be effectively used to remove some of these toxins, however, saxitoxin and its derivatives pose a problem. Little toxicological data are available to evaluate the real threat of these toxins.

Keywords : cyanobacterial, toxin, drinking water, guideline

Introduction

Water is the main constituent of all known forms of life. Even though not uniformly distributed throughout the human body, on average, over 50% of the adult human body is made up of water. An average daily intake of about 2 litres of clean water is essential for the well being of all humans.

A serious threat to the quality of drinking water is cyanobacteria or blue green algae. Cyanobacteria are an integral part of many ecosystems. They have existed for many thousands of million years, much more than any other living organism. From fossil records their earliest existence has been estimated to be over 3 billion years (Schopf 1993, Walter 1993, Carmichael 1994). It is believed that cyanobacteria are the first organism to carry out photosynthesis, thereby also converting atmospheric CO_2 to O_2. These organisms played a major role in providing an oxygen rich atmosphere on prehistoric Earth. Many cyanobacteria are also useful nitrogen fixers and fertilize agricultural land throughout the world, especially rice paddies (Carmichael 1994).

A small group of genera, however, produce toxins. These organisms can bloom in water storage facilities such as water reservoirs and storage dams under the right conditions, with the nutrient content and temperature being the main contributing factors (Bell and Codd 1994). Some of these cyanobacteria have the added advantage over other algae of being capable of fixing nitrogen, hence along with sunlight, only phosphorous and few other trace chemicals are necessary for their existence. Another advantage that some cyanobacteria posses is their capability to adjust their buoyancy within the water column.

The toxins that are produced by cyanobacteria are mostly water soluble and can leach out from the cell into the water body. The presence of these toxins in drinking water is a serious threat to the human health.

Animal deaths due to cyanobacterial poisoning were first reported in 1878 by the Australian Scientist George Francis who reported on the death of livestock that consumed hepatotoxic *Nodularia spumigena* from Lake Alexandrina, South Australia (Francis 1878). Numerous cyanobacterial poisoning incidents, especially involving livestock deaths appear in the literature (Mez *et al.* 1996, Negri *et al.* 1995, Saker *et al.* 1999, Thomas *et al.* 1998). A major human fatality in a renal dialysis centre occurred in a small Brazilian town, Caruaru in 1996. Out of 72 deaths, 52 were confirmed as being caused by cyanobacterial poisoning (Azevedo *et al.* 2002, Carmichael *et al.* 2001, Jochimson *et al.* 1998). Another human fatality incident, involving mainly children, occurred in 1988, also in Brazil when the Itaparica Dam was flooded. This incident is now attributed to cyanobacterial poisoning (Teixeira *et*

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al. 1993). Even though no fatality occurred, a major cyanobacterial poisoning incident took place in 1979 in a small island community in Australia (Bourke *et al.* 1983, Griffiths and Saker 2003). This incident, also known as "Palm Island mystery disease" (Byth 1980) will be discussed later in the article.

Cyanobacterial Toxins

Several species of cyanobacteria are known to produce toxins (Chorus and Bartram 1999). For drinking water purposes, typical toxins produced by these organisms can be broadly classified into two main groups, hepatotoxins and neurotoxins. A third class of toxins, known as endotoxins, is increasingly attracting attention but will not be addressed in this article.

Neurotoxins

Neurotoxins affect the central nervous system. Saxitoxin and their derivatives (about 20) are well known neurotoxins. These toxins are mainly associated with marine diatoms (Red Tide) (Kotaki *et al.* 1983) and related shellfish poisonings. However, *Anabaena circinalis*, a fresh water cyanobacterium which is found in Australia produces saxitoxin and a suite of other similar molecules (Velzeboer *et al.* 2000). These compounds interfere with the transmission of nerve impulses by blocking the sodium channel and interrupting its function, causing respiratory paralysis and ultimately death by oxygen starvation.

Anatoxin-a and antoxin-a(s) are also very potent neurotoxins, both interfering with the signaling pathway of acetylchloline, but in different ways. Both these are produced by different strains of *Anabaena* sp. However, anatoxin-a is produced by several other species, *Oscillatoria*, *Phormidium*, *Aphanizominen* and by *Microcystis* (Codd 2000). Intraperitoneal (IP) LD_{50} of saxitoxin and anatoxin-a(s) on mice are 10 and 50 ug/kg respectively. The structures of these toxins are shown by Fig. 1.

Currently there are no drinking water guidelines for any of these toxins due to the lack of relevant toxicological data.

Hepatotoxins

Hepatotoxins cause major damage to the liver and small intestine (Carmichael 1994, Bell and Codd 1994). There are three main types of hepatotoxins, these are, microcystins, nodularins and cylindrospermopsin. Microcystins are cyclic heptapeptides, produced mainly by *Microcystis* sp., and also by *Anabaena* sp., *Nostoc*, and *Oscillatoria*. Nodularin is a cyclic pentapeptide with a structure similar to microcystin, produced by *Nodularia spumigena*. Cylindrospermopsin is a cyclic alkaloid produced mainly in Australia by *Cylindrospermopsis raciborskii* (Ohtani *et al.* 1992, Shaw *et al.* 2000) and also by *Aphanizomenon ovalisporum* (Banker *et al.* 1997, Shaw *et al.* 1999) and *Umezakia natans* (Harada *et al.* 1994).

Microcystin and Nodularin (The cyclic peptides)

Over 60 different microcystins have been characterized. Most of the structural variations originate from substitution of other amino acids in the positions

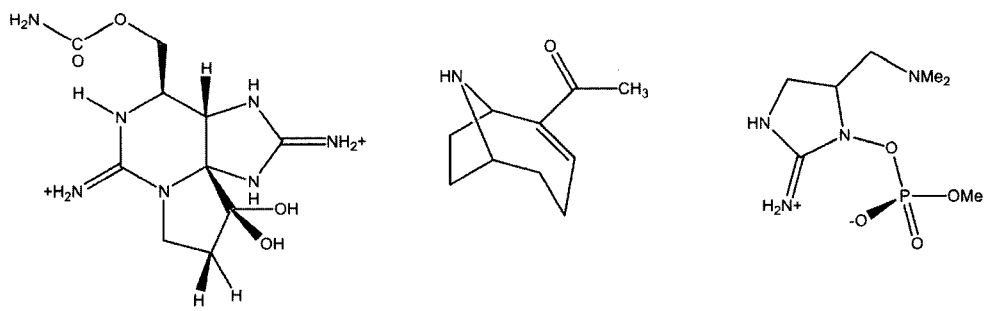

Fig. 1. The structures of Sxitoxin (left), Anatoxin-a (middle) and Anatoxin-a(s) (right).

2 and 4. Microcystin-LR is the most common, here, as shown by Fig. 2, the position 2 and 4 are occupied by the amino acids leucine and arginine respectively. This class of toxins is the most commonly encountered in reticulated water systems and has a worldwide distribution. Nodularin also has the unusual amino acid, Adda, and the structure of nodularin is somewhat similar to microcystin but is a pentapeptide and several structural variants of nodularin are known (Codd 2000).

Toxicology of Microcystins

Microcystins are taken up into the liver through the bile acid transport mechanism (Petzinger 1994). However, there may be other transport mechanisms which are yet to be unraveled. Most of the microcystins are highly water soluble and unable to pass through the lipid membrane directly. However, some microcystins (e.g. MC LA) are hydrophobic and may partition directly across membranes. Microcystin LR is the most toxic isomer discovered to date. Microcystin LA also show similar toxicity.

Microcystins are very potent phosphatase inhibitors. Phosphatases along with protein kinases control many cellular functions through phosphorylation and de-phosphorylation at specific sites. At any given moment there is a subtle balance between the two processes. Over 30% of cellular proteins are subjected to phosphorylation at one or more residues. When phosphatases are inhibited by the action of microcystins, it leads to hyperphosphorylation, rendering many cellular processes inoperative.

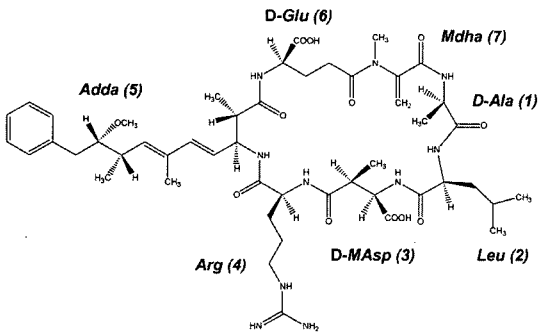

Fig. 2. The structure of microcystin-LR. The numbering sequence of amino acids are depicted in parenthesis.

Acute oral LD_{50} of microcystin LR for mice is 50 ug/kg (Falconer 1993).

The WHO guideline value for microcystin (as LR) in water is 1 ug/*l* (WHO 1998) and the Australian drinking water guideline is 1.3 ug/*l* (as total microcystins) (Australian drinking water guideline 2004).

Cylindrospermopsin

Cylindrospermopsis raciborskii is the major organism responsible for the production of cylindrospermopsin in Australia. Blooms do not usually appear as surface scums like the more well-known toxic cyanobacterial blooms such as *Microcystis*. *C. raciborskii* blooms are dispersed through the upper layers of water bodies (Fabbro & Duivenvoorden 1996) which makes the hazard they pose less visible.

Cylindrospermopsin (CYN) have been associated with fatal incidents of livestock (Pearce & McKenzie 1993, Thomas *et al.* 1998, Saker *et al.* 1999) poisoning and one reported incident of serious human illness (Hawkins *et al.* 1985) in Palm Is. Queensland, Australia.

CYN is a cyclic guanidinium alkaloid containing a uracil moiety. There are three isomers of CYN, two of them are stereo isomers, the only difference is the orientation of the hydroxyl group in position 7 and the other is the deoxy-cylindrospermopsin, where this hydroxyl group is replaced by a hydrogen atom. The structure of cylindrospermopsin and deoxycylindrospermopsin are shown in Fig. 3.

Toxicology of Cylindrospermopsin

According to the current research, all three compounds are toxic (Neumann 2005, Banker *et al.* 2000). Due to the presence of the uracil moiety, these compounds can bind to DNA and have demonstrated genotoxicity (Humpage *et al.* 2000, Shen *et al.* 2002).

When compared to microcystin, CYN is a slow acting toxin, maximum toxicity being realized after about 5 days. Acute intraperitoneal (IP) LD_{50} on mice is 0.2 mg/kg body weight. Acute oral LD_{50} is 6 mg/kg (Seawright *et al.* 1999, Shaw *et al.* 2000). Mechanism of toxicity involves inhibition of protein synthesis (Terao *et al.* 1994, Humpage

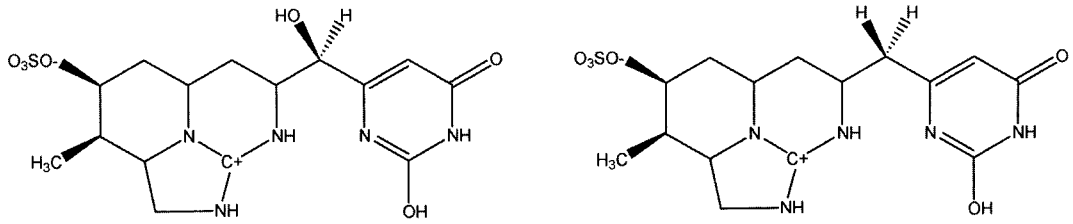

Fig. 3. The structure of cylindrospermopsin (left) and deoxy-cylindrospermopsin (right).

et al. 2000; Shen *et al.* 2002). It appears that a second mechanism of toxicity exists which causes hepatocyte necrosis. Other observations include, pale swollen livers with high level of lipidosis (fatty liver) and also eye lesion. Our current research on cylindrospermopsin is focused on to understand the mechanism of toxicity.

Long term oral toxicity of cylindrospermopsin to mice, No Observed Adverse Effect Level (NOAEL), orally for 90 day trials is about 0.25 mg/kg/day. This is equivalent to a CYN concentration of about 5 mg/*l* in drinking water.

Proposed Drinking Water Guideline Values for Cylindrospermopsin

Our data from dosing mice orally over 90 day period suggests a GV of approximately 10 ug/*l*. Recent investigation by Ian Falconer and Andrew Humpage also suggest a GV of this order (Humpage and Falconer 2003).

Cattle Deaths from Cylindrospermopsin

A number of separate incidents involving cattle deaths have occurred recently in Queensland. Clinical signs were lethargy with subsequent recumbency followed by death within 2-3 days. Autopsy and histopathology showed typical cylindrospermopsin poisoned liver pathology. From the data obtained from these cattle poisoning incidents, we have derived some important toxicological data which would otherwise not have been possible to obtain, since cattle are not a normal test animal for toxicity testing.

Cattle Cylindrospermopsin LD_{50}

Analysis of water and rumen samples showed deaths occurred after consuming water containing 1.05 (3) mg/*l* of CYN. One affected animal had a rumen CYN content of 0.57 (3) mg/*l*). The liver and kidney of affected animals (totaling 4 in this group) had 7.4 (3) to 51 (6) ug/kg and 9.4 (1) to 29 (1) ug/kg of CYN per dry weight basis respectively. No CYN in skeletal muscle were detected. The lowest detection limit of CYN with our instrumentation was 0.2 ug/kg. Values given in parenthesis are the standard error from multiple measurements.

Using the above data as an example, consuming water with a CYN content of 1 mg/*l* for less than 7 days resulted in death of cattle.

Dose
= [CYN] (ug/*l*) × Average Daily Consumption (L) / Body Weight (kg)
= 1000×10 / 250
= 40 ug/kg/day
This amounts to a lethal dose of 40 ug/kg/day.

No NOAEL for cylindrospermopsin for cattle is available, but assuming a similar ratio between LD_{50} and NOAEL for mice and cattle, we could obtain a NOAEL value for cattle.

NOAEL cattle = NOAEL mice × LD_{50} cattle / LD_{50} mice
NOAEL cattle = 250 × 40/6000
NOAEL cattle = 1.7 ug/kg/day

From this data, we can calculate a Tolerable Daily Intake (TDI) level.

TDI = NOAEL/UF, where UF is an uncertainty factor, taking into account, interspecies and intra-species variability and limitations of data for lack of other possible toxic routes.

UF = 10 × 10 × 10 = 1000 (these are standard assumptions made in toxicological work to extend

the data for humans)

Based on cattle data, TDI can be derived as,

TDI = 1.7/1000 ug/kg/day

TDI = 0.0017 ug/kg/day

Based on TDI derived from cattle data, a guideline value for CYN can be calculated as,

GV = TDI × bw × P/C, where P is the fraction of toxin consumed through drinking water, bw is average body weight, C is average consumption of water per day,

GV = 0.0017 × 70 × 1/2 ug/*l*

GV = 0.06 ug/*l*

COMPARISON of GVs (derived from mice and cattle)

GV (from mice) = 10 ug/*l*,

GV (from cattle) = 0.06 ug/*l*

These data suggests that cattle are about 100 fold more susceptible to CYN than mice.

Human epidemiology

In 1979, an outbreak of a "mystery" disease occurred resulting in hospitalization of 148 people, mainly children from Palm Island, Queensland, Australia. The main symptoms were hepatoenteritis. It was subsequently suggested that a toxin from C. raciborskii was responsible (Hawkins *et al.* 1985).

If humans were poisoned by CYN in Palm Is, then we could assume the maximum concentration of CYN in Solomon Dam during the bloom was less than 1 mg/*l*. We arrived at this figure based on culture experiments, where CYN concentration after 3-4 months growth reaches 600-1000 ug/*l* and the fact that reservoirs tested to date normally do not exceed CYN concentrations of 0.1 mg/*l*. The Palm Is incident was an acute poisoning incident. From our experiments described previously, NOAEL for mice is 0.25 mg/kg/day and is equivalent to a CYN concentration of about 5 mg/*l* in drinking water, but cattle die consuming water containing 1.0 mg/*l* of CYN and humans are acutely poisoned consuming water containing 0.1 mg/*l* (or less) of CYN.

It appears that humans may be of similar susceptibility to CYN as cattle and much more susceptible than mice. At least in the case of CYN, the guideline values should not be based on rodent data alone!

This leads us into an interesting, but difficult scenario. What is the alternative model we could use? A quick answer is to use a different species for testing. Pigs for example, Gottingen Minipigs (Ellegarrd 2005) are a good choice. There average weight is about 20 kg. To get a statistically significant Guideline Value, when mice (average weight 20 g) were used as the test animal, for a 90 day trial, about 25-30 mg of cylindrospermopsin was needed. When using a different species with a larger average weight, the toxin needed to be administered will also proportionately go up in weight. In this case by a factor of 1000. This means that to obtain a statistically significant data set using Gottingen Minipigs, about 25-30 grams of cylindrospermopsin would be needed. Currently there are no commercial supplies available and the cost of cylindrospermopsin is about $1000 /mg.

Removal of Cyanobacterial Toxins from Drinking Water

Most of the cyanobacterial toxins, if managed well, can effectively be removed from drinking water, either by chlorination, ozonation or by photocatalytic oxidation in the presence of a suitable photocatalyst like TiO_2. However, when present, the removal of saxitoxin and derivatives pose a problem to water authorities. Under normal conditions (pH 7-8) these toxins are difficult to oxidize by chlorine. To remove these toxins, chlorination at elevated pH (pH>9.0) is necessary. Our recent studies have also shown that saxitoxin and derivatives can be removed effectively and efficiently from drinking water by photocatalytic oxidation using TiO_2. The pH does not seem to have much effect when this oxidation path is chosen.

Conclusion

Drinking water safety is a major concern to everyone, since it is a lifetime affair. Human populations will always be at risk where toxic algal blooms are concerned. It is possible that poisoning episodes such as Caruaru and Palm Is. may occur in future. However, this risk can effectively be managed by better understanding the problem with a multifaceted approach. For this to be

successful, ecologists, water managers, toxicologists, analytical chemists and policy makers must work with hand in hand. As the research progress and with a better understanding of these toxins, it is expected that guideline values be set for the toxins which are under study and be continually revised with the availability of better data.

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