Session I-2

Cyanobacterial Toxins and Drinking Water Guidelines

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Abstract

The occurrence of toxic cyanobacterial blooms has been reported worldwide and pose a threat to human health through drinking water exposure. The toxins they produced 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 derivatives pose a problem. Little toxicological data are available to evaluate the real threat of these toxins.

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, there by also converting atmospheric CO_2 to O_2. These organisms played a major role in providing an oxygen rich atmosphere on primitive Earth. Many cyanobacteria are also useful nitrogen fixers and fertilize the 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 as they are capable of fixing nitrogen, hence along with sunlight, only phosphorous and few other trace chemicals are

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necessary for their existence. Another advantage that some cyanobacteria posses is their capability to adjust the 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 1996, Negri 1995, Saker 1999, Thomas 1998). A major human fatality in a renal dialysis centre occurred in a small Brazilian town, Caruaru in 1996. Out of 72 deaths, 52 confirmed caused by cyanobacterial poisoning (Azevedo 2002, Carmichael 2001, Jochimson 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 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). 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.

Endotoxins

There is some misunderstanding about the term "Endotoxins", these are the lipopolysaccharides of the bacterial cell walls. One adverse effect of some lipopolysaccharides, when in contact with skin is to give skin rashes, hence they are also known as dermal toxins. These toxins however, have also been attributed to other illnesses such as gastrointestinal, respiratory and allergic reactions (Stewart 2004).

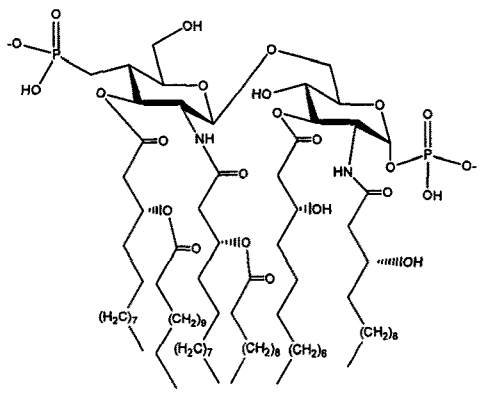

Figure 1. The structure of a gram negative Lipopolysaccharide.

The three dimensional structure of a cyanobacterial lipopolysaccharide has yet to be determined, but these are believed to have similar structures to gram negative bacterial lipopolysaccharides. The structure of such a lipopolysaccharide molecule is shown by the figure 1. These molecules have long aliphatic chains attached to a phosphorylated sugar molecule and are hydrophobic at one end and hydrophilic at the other end. The exact mechanism how these molecules function as irritants are not yet known.

Currently there are no guideline values associated with this class of toxins.

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 1983) and related shellfish poisonings. However, *Anabaena circinalis*, a fresh water cyano-bacterium which is found in Australia produces saxitoxin and a suite of other similar molecules (Velzeboer 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.

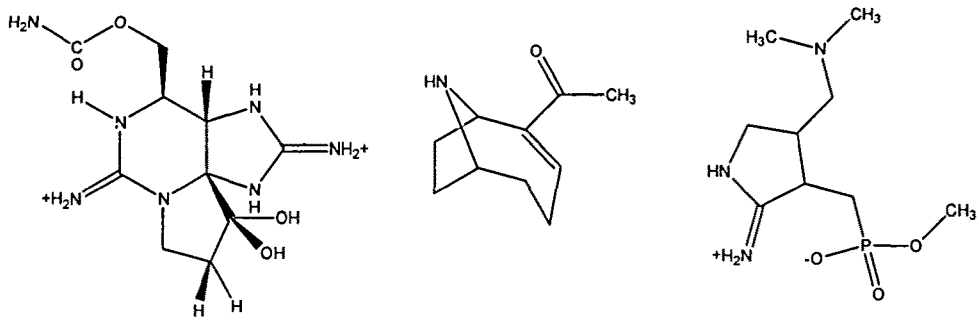

Figure 2. The structures of Sxitoxin (*left*), Anatoxin-a (*middle*) and Anatoxin-a(s) (*right*).

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 *Anabena 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.

Currently there are no drinking water guide lines 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 1994]. There are three main types of hepatotoxins, these are, microcystins, nodularins and cylindro-spermopsin. 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 1992, Shaw 2000) *and also by Aphanizomenon ovalisporum* (Banker 1997, Shaw 1999) *and Umezakia natans* (Harada 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 2 and 4. Microcystin-LR is the most common, here, as shown by figure 4, the position 2 and 4 are occupied by the amino acids leucine and arginine respectively. This class of toxins are the most commonly encountered in reticulated water systems and has a world wide distribution. Nodularin also has the unusual amino acid, Adda, the structure is somewhat similar to microcystin but is a pentapeptide and several structural variants of nodularin are known (Codd 2000).

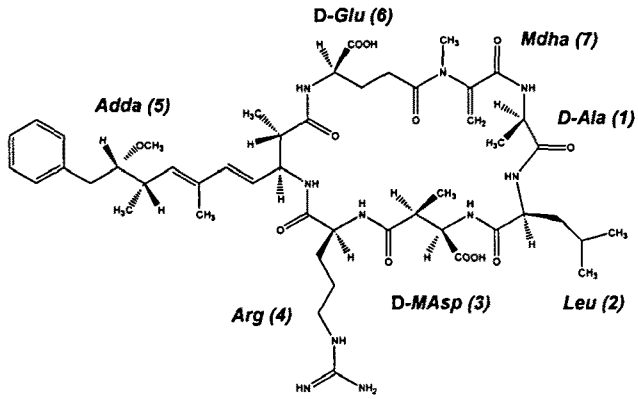

Figure 3. The structure of microcystin-LR. The numbering sequence of amino acids are depicted in parenthesis.

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.

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

WHO guideline value for microcystin in water is 1 ug/L (WHO 1998) and the Australian drinking water guideline is 1.3 ug/L (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 like the those of *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 1998, Saker1999) poisoning and one reported incident of serious human illness (Hawkins 1985) in Palm Is. Queensland, Australia.

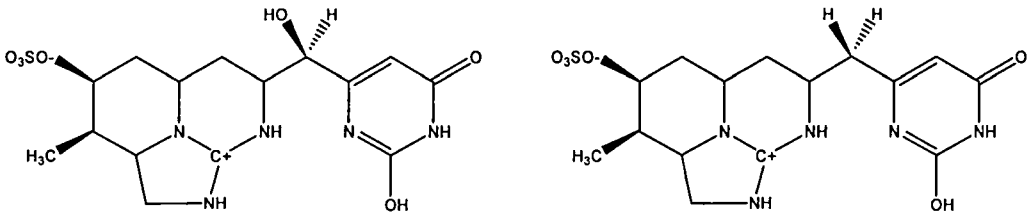

Figure 4. The structure of cylindrospermopsin (*left*) and deoxy-cylindrospermopsin (*right*)

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.

Toxicology of Cylindrospermopsin

According to the current research, all three compounds are toxic (Caroline 2005, Banker 2000). Due to the presence of the uracil moiety, these compounds can bind to DNA and have demonstrated genotoxicity (Humpage 2000, Shen 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 1999, Shaw 2000). Mechanism of toxicity involves inhibition of protein synthesis (Humpage 2000, Shen 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 0.25mg/kg/day. This is equivalent to a CYN concentration of about 5mg/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 10ug/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 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) x Average Daily Consumption (L) / Body Weight (kg)
= 1000 x 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 x LD_{50} cattle / LD_{50} mice
NOAEL cattle = 250x40/6000
NOAEL cattle = 1.7 ug/kg/day

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

TDI = NOAEL / UF, where UF is an uncertainty factor, taking into account, interspecies and intraspecies variability and limitations of data for lack of other possible toxic routes. UF = 10x10x10 = 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 cattle TDI, a guideline value for CYN can be calculated as,
GV = TDI x bw x 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 x 70 x 1/2 ug/L

GV = 0.06 ug/L

COMPARISON of GVs (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 of Cylindrospermopsin
In 1979, an outbreak of a "mystery" disease occurred resulting in hospitalization of 148 people, mainly children from Palm Island, Queensland, Australia. Main symptoms were hepatoenteritis. It was subsequently suggested that a toxin from *C. raciborskii* was responsible (Hawkins 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 1mg/L. We arrived this figure based on culture experiments, where CYN concentration after 3 -4 months growth reaches 600 - 1000 ug/L. The Palm Is incident was an acute poisoning incident. This equates to a dose of 0.25 mg/kg/day for which is around the NOAEL for mice, but we have already shown that this dose is highly toxic to cattle.

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, Göttingen 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 Göttingen 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 can 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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Cyanobacterial Toxins, Drinking Water and WHO Guidelines

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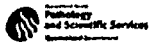


Cyanobacteria are an integral part of many ecosystems.

They have been around for a long time, much more than any other living organism.

From Fossil records > 3.0 billion years.

First organism to carry out Photosynthesis.

$CO_2 \longrightarrow O_2$

Played a major role in providing an Oxygen rich atmosphere on Earth.

Also useful nitrogen fixers.

Fertilize agricultural land throughout the world.

A small group of genera, however, produce toxins. These toxins affect human health and livestock.

Animal deaths were first reported in 1878 (Francis).

1988 Brazilian incident (Itaparica Dam). 88 deaths caused by unknown cyanobacterial toxins. Didn't get much publicity.

1996 Incident. Out of 72 deaths, 52 confirmed caused by cyanobacterial poisoning.

Brazilian incident in 1996.

Brazilian City of Caruaru, A Renal Dialysis Centre.

13th February, 1996 - People receiving routine renal dialysis treatment began to complain about, headaches, eye pain, blurred vision and nausea.

20th February, one patient died suddenly. Over the next few months 76 more patients died from acute liver failure.

Brazilian incident in 1996.

Samples of liver, serum and also the clinic's water source and purification system were later analyzed.

Estimated microcystin content used in dialysis water was 19.5 ug/L

The water was obtained from a local dam, carted to the hospital by truck, filtered, treated with ion exchangers and carbon, but not disinfected by chlorination or other method.

Many of the world's water resources are subjected to increasing levels of nutrients.

It is *highly probable* that repeat episodes of cyanotoxin poisoning may occur in future.

At the *National Research Centre for Environmental Toxicology,* Natural Toxins Research is primarily focused on freshwater cyanobacterial toxins and their toxicology.

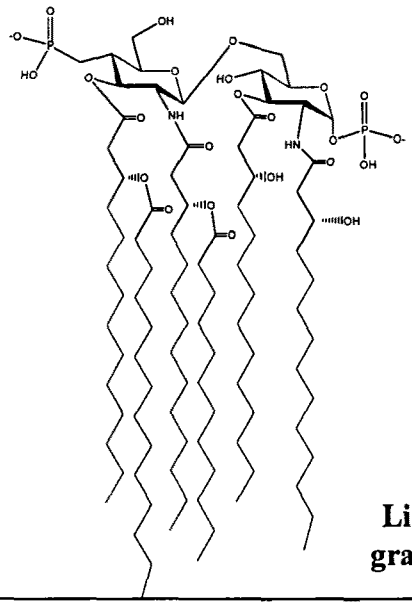
Main objective is to understand human health implications of these toxins.

What are these toxins?

Cyanobacterial toxins are a chemically diverse group of compounds.

These toxins are commonly classified as,

- **Endotoxins (Lipopolysaccharides)**
- **Neurotoxins (Alkaloids)**
- **Hepatotoxins (Cyclic peptides and Alkaloids)**

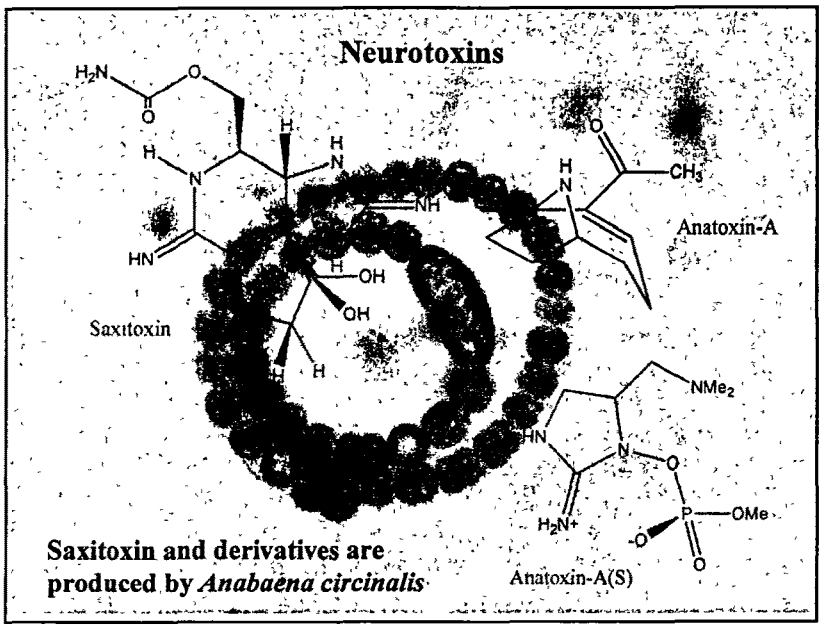


Detailed structure of a cyanobacterial lipopolysaccharide has not being determined.

Structure is assumed to be similar to gram negative lipopolysaccharides (?).

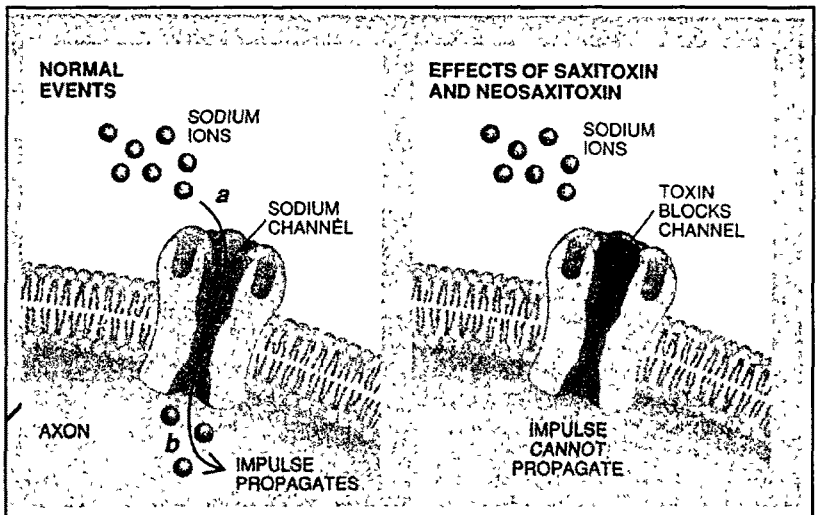
These compounds may be responsible for respiratory, gastrointestinal and allergic reactions.

Lipid A structure of a gram negative bacteria.

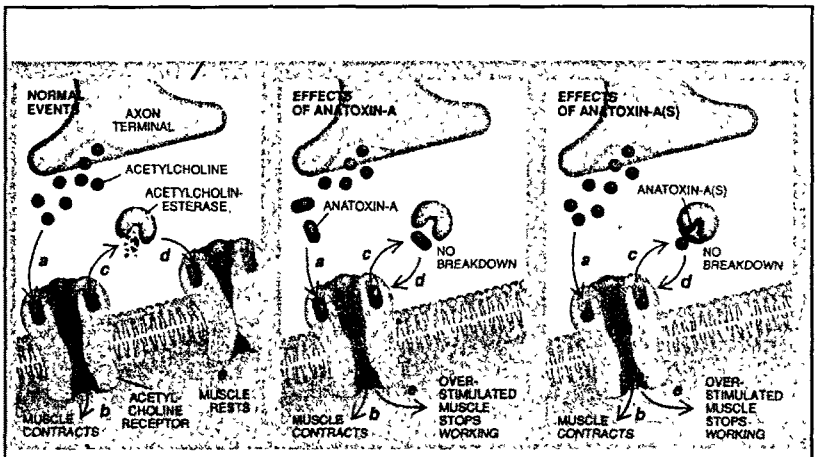


COMPARATIVE TOXICITIES

TOXIN	SOURCE	LD 50 ug/kg
Botulinum toxin-a	*Clostridium botulinum*	0.00003
Ricin	Castor bean plant	0.02
Tetrodotoxin	Puffer fish	8
Saxitoxin	*A. circinalis*	9
Anatoxin-a(s)	*A. flos aquae*	20
Nodularin	*N. spumigena*	50
Microcystin LR	*M. aeruginosa*	50



Saxitoxin blocks the Sodium Channel



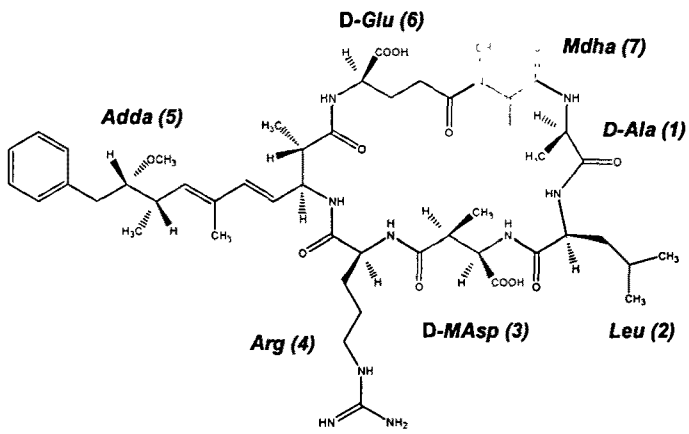
Anatoxin-a and Anatoxin-a(s) disrupt *Acetyl-Choline receptor* by different mechanisms.

Hepatotoxins

Cause major damage to the liver.

There are three main types of hepatotoxins,

- **Microcystins**
- **Nodularins**
- **Cylindrospermopsin**



Microcystin-LR, cyclic heptapeptides

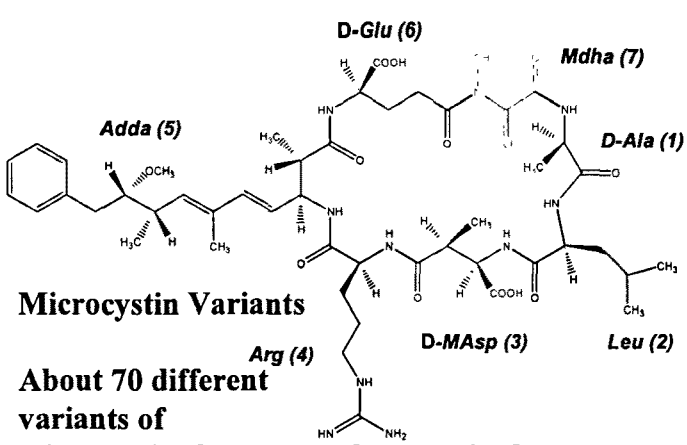
SOURCES OF MICROCYSTINS

Microcystins are produced by a number of cyanobacteria.

Main source is *Microcystis aeruginosa.*

Other genera include: *Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis.*

Nodularin is produced by *Nodularia spumigena.*



Microcystin Variants

About 70 different variants of microcystins have been characterized. Recently, we have isolated 4 unknown variants, the structures of these are currently being elucidated.

Uptake of microcystins and nodularin

They are taken up into the liver through the bile acid transport mechanism.
There may be other transport mechanisms.

Most of these microcystins are highly water soluble and unable to pass through lipid membrane directly.

Some microcystins (eg. MC- LA) are hydrophobic and may partition directly across membranes.

TOXICOLOGY OF MICROCYSTINS

Microcystin LR (leucine/arginine) is the most toxic isomer discovered to date.

Microcystin LA also show similar toxicity.

ADDA moiety is essential for toxicity.

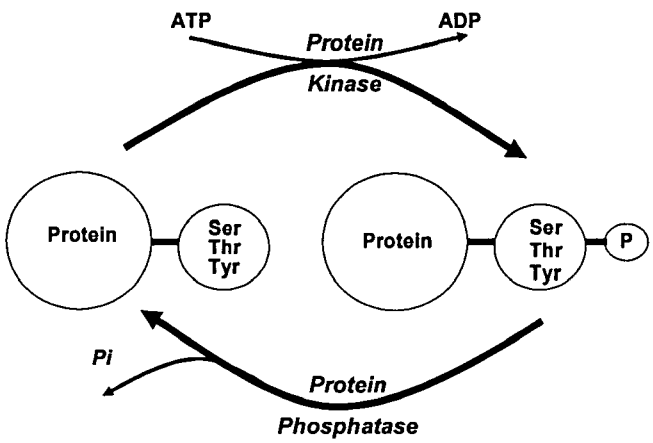
Acute oral LD_{50} for mice is 50 ug/kg

MECHANISMS OF TOXICITY OF MICROCYSTINS

Toxicity is caused by binding to cellular enzymes, protein phosphatases.

The binding inhibits the action of the phosphatases.

Microcystins are also Tumour Promoters!



Protein Phosphatase in Signal Transduction

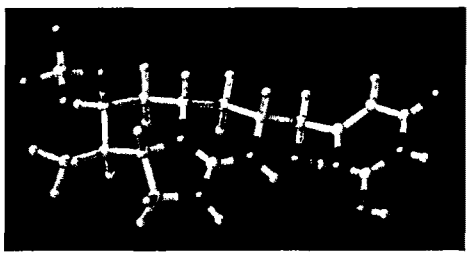
Many cellular processes are controlled by phosphorylation and dephosphorylation of proteins at specific sites.
This is a well regulated process.

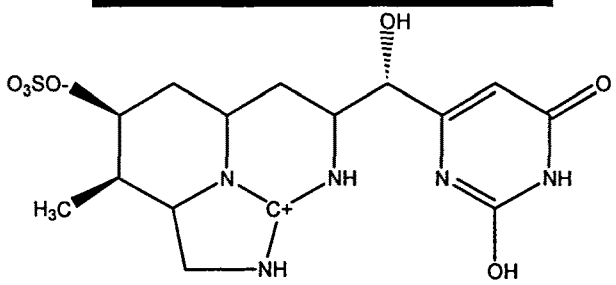
WHO guideline value for microcystin in water is 1 ug/L

Australian guide line value is 1.3 ug/L

Taking into account both the toxicity and tumor-promoting properties, a value of 0.3 ug/L has been suggested.

CYLINDROSPERMOPSIN

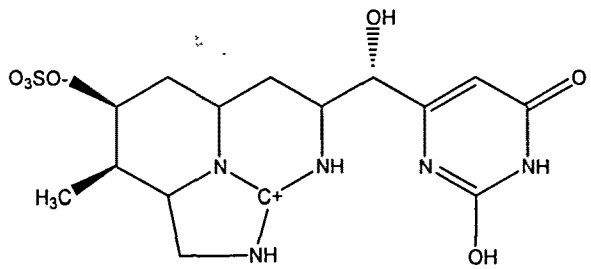




Cylindrospermopsin is a relatively new (1992) toxin.

Produced mainly by, ***Cylindrospermopsis raciborskii***

Dominant toxigenic cyanobacterium in Queensland, Australia



Cylindrospermopsin has 2 stereo isomers.

Also, deoxycylindrospermopsin.

All three compounds are toxic.

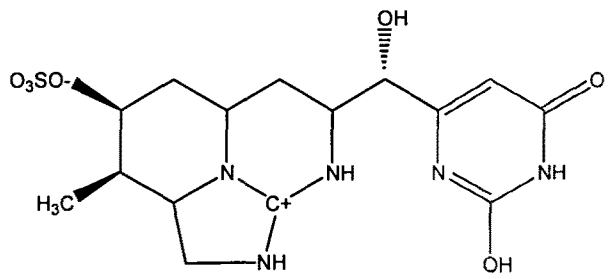
Cylindrospermopsin is a liver toxin.

Acute IP LD_{50} for mice = 0.2 mg/kg
Acute oral LD_{50} = 6 mg/kg

Mechanism of toxicity involves inhibition of protein synthesis.

It appears that a second mechanism of toxicity exists which causes hepatocyte necrosis.

Cylindrospermopsin could also be genotoxic.



Current research on Cylindrospermopsin is focused on to understand the mechanism of toxicity.

Long term oral toxicity of cylindrospermopsin to mice (for 90 days):

No Observed Adverse Effect Level (NOAEL)

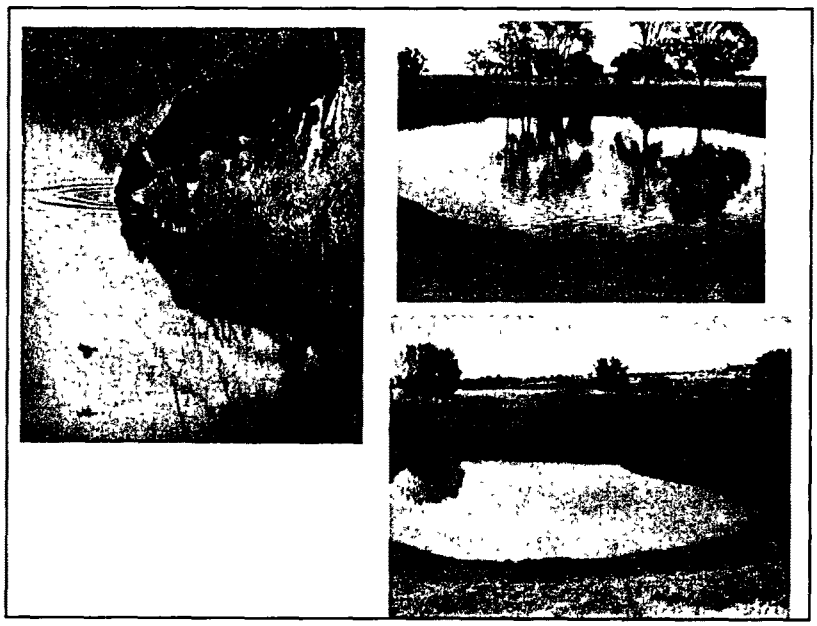
Orally is about 0.25mg/kg/day.

Equivalent to up to 5mg/L in drinking water for mice.

PROPOSED GUIDELINE VALUES FOR CYLINDROSPERMOPSIN IN DRINKING WATER (based on MOUSE DATA)

Our data suggests a Guideline Value (GV) of approximately 10 μg/L

Recent investigation by Ian Falconer and Andrew Humpage also suggest a GV of this order.



CATTLE -CYLINDROSPERMOPSIN LD_{50}

Drinking water at 1mg/L for less than 7 days results in death of cattle.

Dose = Conc of Toxin (ug/L) x daily intake /bw
= 1000 x 10/250

Lethal dose = 40 ug/kg/day

No Observed Adverse Effect Level (NOAEL) for cylindrospermopsin from cattle data

No NOAEL available

Assume similar ratio between LD_{50} and NOAEL for mice and cattle

NOAEL cattle = NOAEL mice x LD_{50} cattle / LD_{50} mice

NOAEL cattle = 250x40/6000
NOAEL cattle = 1.7 ug/kg/day

COMPARISON, NOAELs & GVs (mice and cattle)

NOAEL mice = 250 ug/kg/day
NOAEL cattle = 1.7 ug/kg/day

Tolerable Daily Intake (TDI)
TDI = NOAEL/UF

UF is an uncertainty factor, taking into account, interspecies and intraspecies variability and limitations of data for lack of other possible toxic routes.

UF = 10x10x10 = 1000 (these are standard assumptions made in toxicological work to extend the data for humans)

TDI = NOAEL/UF

GV (from mice) = 10 ug/L
GV (from cattle) = 0.06 ug/L

QUESTION: ARE HUMANS MORE SIMILAR TO MICE OR CATTLE?

WHICH GV SHOULD WE USE?

Human epidemiology.

In 1979, an outbreak of a "mystery" disease occurred resulting in hospitalisation of 148 people from Palm Island, Queensland, Australia.

Main symptoms were hepatoenteritis.

It was subsequently (1985) suggested that a toxin from *C. raciborskii* was responsible.

Human epidemiology.

If humans were poisoned by Cylindrospermopsin in Palm Is, then we would assume the maximum concentration in Solomon Dam was 1mg/L of toxin.

This was an acute poisoning incident.

This dose is highly toxic to cattle.

HUMAN HEALTH IMPLICATIONS

It appears that humans may be of similar susceptibility to cylindrospermopsin as cattle, and much more susceptible than mice.

Guideline values should not be based on rodent data alone!

What is the alternative?

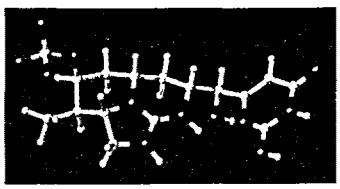
Use a different species for testing.

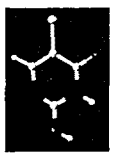
Pigs are a good choice.
Göttingen Minipigs, weight 20 kg

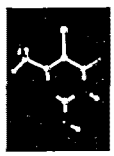
To get a statistically significant Guideline Value, need a large amount of toxin.

Currently no commercial supplies available;
Cylindrospermopsin cost about 1000 $/mg

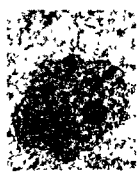
For a 90 day trial, using 20 g mice (avg. weight), need about 25 – 30 mg of toxin.







Possible byproducts of partial oxidation of Cylindrospermopsin.



Electron micrographs of mouse liver hepatocites which show nuclear changes induced by chlorinated uracils.

Removal of cyanobacterial Toxins from Drinking Water

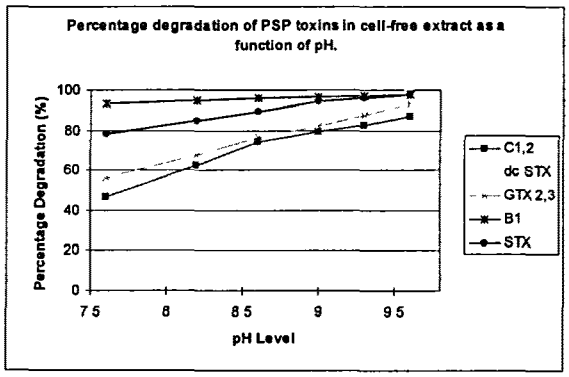
Most widely practiced disinfection technique in water industry is Chlorination.

If managed well, Microcystins and Cylindrospermopsin can be effectively and efficiently removed from drinking water by Chlorination.

Minimum of 0.5 mg/L chlorine level must be maintained for a period of 30 minutes.

Standard Chlorination techniques will not remove saxitoxin and derivatives from drinking water.

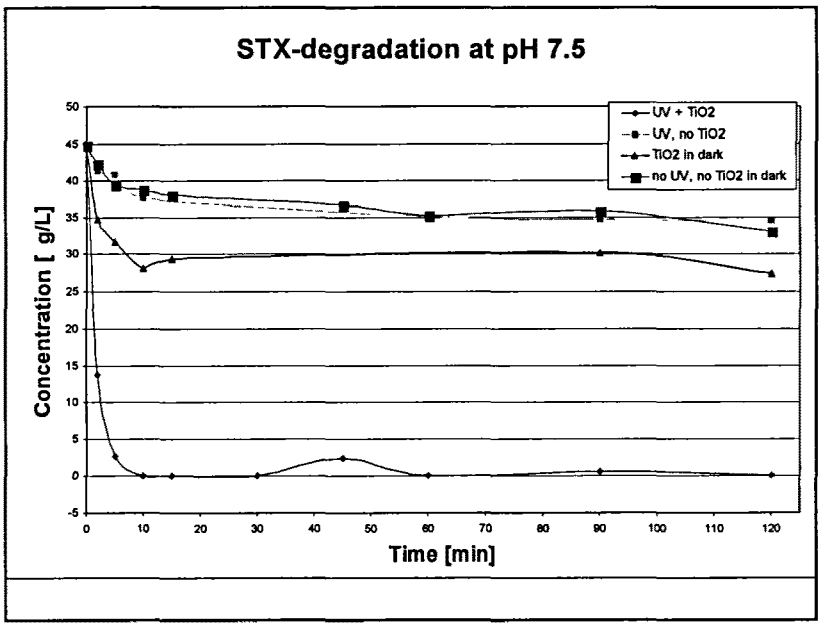
Chlorination at elevated pH (9.0 and above) remove the toxin.



Chlorination of PSP Toxins at Various pH levels

Raising and lowering of pH in a water utility is an expensive process.

This led us to investigate alternative techniques that can be used by the water industry.



Survival Techniques of *Cylindrospermopsis raciborskii* is due in part to the organism's ability to produce reproductive spores (*akinetes*).

To understand the life cycle of this organism, the factors that control the formation and growth of akinetes have been studied.

These are, changes in temperature, light conditions and phosphorus availability.

Improve our capacity to manage this toxic species. (Dave Moore)

Effects of climate change on toxin production *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* (Corinne Garnet).

Toxicity of cylindrospermopsin producing cyanobacteria, *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* to aquatic biota is largely unknown.

Aim is to generate new and essential information on the toxicity of cylindrospermopsin to a range of aquatic organisms.

Has widespread implications for reservoir management and monitoring (Marc Seifert, David Rubhart).

Acknowledgement

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