

I. Microcystin TOXICITY

History

The earliest report of cyanobacteria poisoning may have been about 1,000 years ago when General Zhu Ge-Ling reported mortality in troops that drank water from a river in southern China that was green (1). The first known reported incidence of cyanobacteria toxin poisoning was from an Australian lake in 1878 (2). With increasing eutrophication of lakes due to increased agricultural use and population pressures, the number of cyanobacteria blooms appears to be increasing. A high incidence of primary liver cancer in China has been attributed to cyanobacterial contaminated drinking water (3) and contamination of water supplies for dialysis units recently resulted in 60 deaths in Brazil (4).

Human incidences of gastroenteritis have also been reported to be associated with algae blooms in Pennsylvania, Virginia, New Jersey and Washington, DC, in some cases involving thousand of individuals (5, 6). A World Health Organization (WHO) in a guideline for drinking water quality identified cyanobacteria as one of the most urgent areas in which guidance was required (1). The WHO guideline for microcystins in finished drinking water is 1 µg/L (ppb), based on microcystin-LR, a specific microcystin toxin.

Taxonomy

Blue-green algae of Cyanobacteria are ancient and remarkable organisms that inhabit very diverse environments including hot springs, arctic lakes, snow and infertile substrates such as volcanic ash, desert sand and rocks (1). The Cyanobacteria are primitive microalgae with plant chlorophyll and include nearly 2000 species. Cyanobacteria as prokaryocytes lack a nucleus (7). They grow as single cells, single cells in colonies or single cells in filaments. Increasing levels of nitrogen, phosphorus, temperature, light and other nutrients can lead to the occurrence of algae blooms in source water. *Microcystis aeruginosa*, *Microcystis viridis*, *Aphanizomenon flos-aquae*, and *Anabaena species* are all associated with microcystin, the most common toxin found with cyanobacteria (7). In a survey of 167 water samples in Florida, *Microcystis* (43%), *Cylindrospermopsis* (40%) and *Anabaena* (29%) were observed most frequently (8). These cyanobacteria also occurred at greatest concentrations in the waters sampled (8). In the same report, forty-seven of the samples (28%) were lethal to mice when up to 1 mg (dry weight, lyophilized sample) were injected per mouse.

The algae blooms may be dominated by a single species or composed of multiple species. Even within a single species there may be a mixture of toxic and non-

toxic strains (1). *Microcystis* species, most commonly *Microcystis aeruginosa*, are most frequently associated with the algae blooms associated with hepatotoxicity (1, 7).

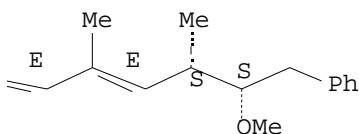
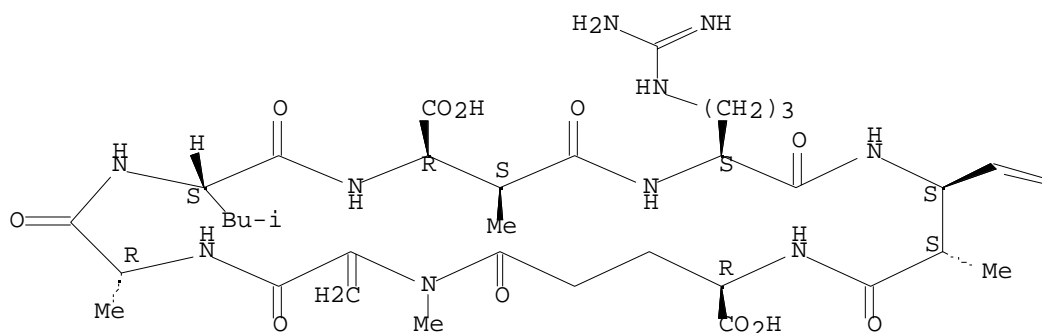
Fifty samples taken from lakes human water supplies, farm waters and aquaculture facilities were all positive for *Microcystis aeruginosa* (9). With another Cyanobacteria outbreak in Brazil that caused mortality in a dialysis unit, *Anabaena* and *Microcystis* genera were present in the water at 1,104 and 9,755 units (units refers to colonies which could be up to 100 cells) per ml (4). In Florida surface waters *Cylindrospermopsis* species counts exceeded 8,000 cells/ml for several sites during the summer months (8).

Health food supplements harvested from natural blooms in Oregon were found to contain as high as 20 µg of Microcystin LR per gram of supplement (10).

Cyanobacterial toxins

The cyanotoxins are a very diverse group of toxins from both the chemical and toxicology standpoint. Toxicities include neurotoxicity, hepatotoxicity, cytotoxicity and dermatotoxicity. The microcystins are the most common algae toxin found and are associated with *Microcystis*, *Anabaena*, *Oscillatgoria*, *Nostoc*, *Hapalosiphon* and *Anabaenopsis* species. The microcystins are generally associated with hepatotoxicity (1). The microcystins are cyclic heptapeptides with variable amino acids at 7 different positions. The name microcystin derived from the toxins that were first isolated from *Microcystis aeruginosa*. The toxicity of microcystins is due to their strong binding to protein phosphatases (1, 7, 11, 12).

Currently many water utilities are concerned about controlling odor and taste and may not fully appreciate the potential consequences of long-term low concentration exposure. With the diversity of toxins, it would appear prudent to focus first on microcystin, the toxin that occurs most frequently in surface water supplies. There are a variety of microcystins that vary slightly but a standard and common microcystin is Microcystin LR (9CI) (RN 101043-37-2). Microcystin is sometimes known as CN Toxin I (*Microcystis aeruginosa*) or Toxin T 17 (*Microcystis aeruginosa*) with a chemical name of Cyclo[2,3-didehydro-N-methylalanyl-D-alanyl-L-leucyl-erythro-3-methyl-D-beta.-aspartyl-L-arginyl-(2S,3S,4E,6E,8S,9S)-4,5,6,7-tetrahydro-9-methoxy-2,6,8-trimethyl-10-phenyl-3-aminodecanoyl-D-.gamma.-glutamyl]. The structure is as follows:



Toxicity of Cyanobacterial toxins in humans

Many of the reported adverse human effects from cyanotoxins derive from epidemiology studies or antidotal reports from poisonings. Not all reports carefully define the organism and especially the toxin since only recently have analytical procedures been available for these complex toxins. In 1931, low rainfall caused the water in a side branch of the Ohio River to develop a cyanobacterial bloom that was then washed into the main river. As the bloom washed down the river, a series of gastroenteritis outbreaks occurred which could not be attributed to infectious disease (1). In Harare, Zimbabwe, gastritis in children drinking water from a reservoir coincides with a *Microcystis* bloom each year and in Brazil 88 deaths were reported to be associated with cyanobacterial toxins (4).

In Armidale, New South Wales Australia repeated blooms of *Microcystis aeruginosa* have been documented since the 1970's. In 1981, an extensive bloom was associated with increased serum enzymes consistent with hepatotoxicity (1). An investigation into severe hepatitis in a dialysis unit with 50 deaths in Brazil in 1996 revealed microcystins from the dialysis unit's intake filters (1). Serum and sera from the deceased patients, analyzed at the CDC in Atlanta were positive for microcystins. Very little is known about the chronic low dose effects of microcystin exposure. In China, the highest incidence of liver cancer occurs in areas with abundant cyanobacteria in the surface waters (1). The WHO has noted

that these cyclic peptides represent the greatest concern to human health because of their potential exposure to low concentrations for long-periods of time.

Toxicity of Cyanobacterial toxins in *in vitro* studies

Microcystin LR is a potent inhibitor of the protein phosphatases 1 (PP-1) and PP-2A (12) *in vitro* but has no effect on protein kinase C or cyclic AMP-dependent kinase (11). Microcystins appear to act similar to other alkaloid promoters through PP-1 and PP-2A in contrast to phorbol esters which bind to and activate protein kinase C (13). Mutagenicity has not been observed for purified toxins derived from *Microcystis* but were clastogenic for human lymphocytes (1). Extracts from *Microcystis aeruginosa* did not cause transformation of Syrian hamster embryo (SHE) cells but when SHE cells were first initiated with methylcholanthrene, an increased transformation was observed (1).

Toxicity of Cyanobacterial toxins in animal studies

Microcystins

After intravenous or intraperitoneal injections, microcystins localize in the liver (14, 15). It does not readily cross cell membranes and the localization in the liver appears to be the result of active uptake by hepatocytes (1). The microcystins are highly toxic with 25 to 150 µg/kg body weight given orally or intraperitoneally lethal to mice (1). Perhaps due to poor absorption the oral doses are much less toxic requiring 5 to 10 mg/kg body weight for lethality in mice (1). The microcystins are hepatotoxic (16) causing hepatic necrosis within 60 minutes of an intravenous dose (17).

Extracts of the bloom of *Microcystis aeruginosa* did not cause increased tumors rates in groups of mice treated for up to one year (16). The small group size (40) and treatment for only one year may have diminished the sensitivity of the assay. In another study, mice given 20 mg/kg body weight approximately 4 times per week for 28 weeks developed neoplasms of the liver (18).

In a report by Fawell et al., cited in a book on cyanobacteria (1), microcystins do not appear to show developmental toxicity.

Proposed Studies

The WHO guidelines for microcystins based on microcystin LR are 1 µg/L for finished water. With improving analytical techniques, detection of the microcystins, often above the 1 µg/L are found in surface waters. It is crucial to determine the effects long-term exposure to low concentrations of microcystins in

the drinking water. This need was also recognized by the WHO cyanobacteria (1). Microcystin LR is recommended for consideration since this is one of the most common cyanobacteria toxins found in US waters.

Since toxicokinetic studies are limited for microcystin LR, TK studies may be indicated. It is recommended that 14 and 90 day studies are used to select the doses for the long-term studies. The studies should include the F344 rat and the B6C3F1 mouse.

Cancer is considered a high priority with the emphasis on hepatocellular cancer. Microcystins are reported to act as okadaic acid class of tumor promoters through inhibition of protein phosphatases 1 and 2A (13). Therefore additional studies using initiator/promoter rodent models may be indicated.

The focus should be on low concentrations. The microcystins may cause mortality and hepatotoxicity in rodents at parenteral administrations of 5 to 10 mg/kg body weight. In a survey of Florida lakes concentrations of less than one µg/L to greater 100 mg/L were found. The concentrations in the finished drinking water would be less. The human incidences of cyanobacteria toxicity and the ecotoxicity have focused almost entirely on the acute episodes. There is a crucial need for chronic drinking water toxicity studies. With diminishing clean water supplies, increasing eutrophication of surface waters and global climate changes, cyanobacteria toxicity are emerging issues. While microcystin LR is a logical first toxin for evaluation, *Cylindrospermopsis* and *Anabaena* toxins should also be considered.

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