FRI FOOD SAFETY REVIEWS

TOXINS FROM CYANOBACTERIA

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Contents

Cyanobacteria (previously called blue green algae) are ancient single-celled organisms, widely distributed in aquatic environments, soil, and other moist surfaces and can survive in very inhospitable environments such as hot springs and the arctic tundra. Some species partner with fungi to form lichens, and others engage in symbiotic relationships with higher plants. Cyanobacteria in lakes are often overlooked until their populations grow rapidly, forming blooms that cover large areas of the surface of lakes. Increased light and warm temperatures of spring and summer and high nutrient levels, particularly phosphorus in runoff from agricultural operations and lawn fertilizers, are known to stimulate growth of cyanobacteria.

Many cyanobacteria produce toxic compounds, presumably for self-defense. Toxin production is strain-specific and is influenced by environmental factors. These toxins include:

- Microcystins: heat stable, cyclic heptapeptides that are toxic to liver cells and promote growth of some tumors
- Beta-methylamino-L-alanine (BMAA): a neurotoxin, possibly associated with amyotrophic lateral sclerosis and Alzheimer's Disease
- Paralytic shellfish poisoning toxins: neurotoxins that block sodium channels in neurons and calcium and potassium channels in cardiac cells
- Anatoxin-a: neurotoxin that inhibits acetylcholinesterase

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Intake of contaminated drinking water or food is the main route of human exposure to cyanotoxins. Humans may be exposed to these toxins by intentional consumption of cyanobacteria. In some traditional communities in Asia and South America, globular colonies of *Nostoc commune* are harvested from lakes or rice paddies and are eaten alone or in soups or stews (*21;36*). There have also been numerous reports of healthy compounds in cyanobacteria, and extracts of cyanobacteria have become popular as dietary supplements (*8;33*). Cyanotoxins may also be present in drinking water derived from surface water sources and in food, particularly fish and shellfish that have consumed cyanobacteria. Exposure also occurs from aerosols from lakes with cyanobacterial blooms and during recreational activities in such lakes (*43*). Risks to human health from different routes of cyanotoxin exposure were reviewed with a discussion of both acute, short term effects and chronic, long term effects (*1;14*). Another recent review discussed inactivation and removal of cyanotoxins during drinking water treatment (*42*). An earlier FRI article on microcystins and a toxic alga, *Pfiesteria*, is appended to this review.

Microcystins

Microcystins are heat-stable, water-soluble, cyclic peptides, containing 7 amino acids, that are produced by some strains of several genera of cyanobacteria: *Microcystis*, *Anabaena*, *Nostoc*, and *Oscillatoria*. Over 70 varieties of microcystins have been described. These toxins are usually contained within living cells but are released when cells die or are lysed by water treatments, such as copper sulfate. Microcystin synthesis appears to peak at high cell concentrations, and total microcystin concentrations of several mg/L have been reported in some scum samples (*18;44*).

Microcystins irreversibly inhibit serine/threonine phosphatase enzymes, causing liver and kidney damage. Acute liver failure resulting in 56 deaths occurred in Brazil in 1996 when water containing microcystin was used for hemodialysis (*20*). Although some research reports indicate that microcystins may have carcinogenic effects, IARC reviewed these studies and concluded that available data indicate that these toxins should be classified as possible carcinogens for humans and other animals (*18;46*).

WHO has set a provisional tolerable daily intake of 0.04 µg microcystin/kg body weight and a recommended guideline limit of 1 µg/L in drinking water. Microcystins have been detected in drinking water samples from many countries, but toxin levels are usually well below the guideline and acute oral doses are unlikely to occur. However, some communities consuming inadequately treated surface waters may be chronically exposed to elevated microcystin levels (*34*). When particles are removed during water treatment, most microcystins are also eliminated along with the cyanobacterial cells. Toxins released from damaged cells can be neutralized by oxidation with chlorine or ozone (*18*). Several drinking water treatment processes and biodegradation by certain bacteria can significantly decrease microcystin levels in water (*11;24;42*).

Humans may also be exposed to microcystins by eating animals that have themselves consumed cyanobacteria or cyanotoxins in water. Analyses for microcystins in muscle tissue of several species of fish revealed that walleye (23.9 µg/kg wet weight), white bass (18.3 µg/kg wet weight), and smallmouth bass (13.4 µg/kg wet weight) in Lake Erie and alewives (25.9 µg/kg wet weight) and northern pike (10.2 µg/kg wet weight) in Lake Ontario contained the highest average levels. The highest level detected in any fish from these lakes was 43.6 µg/kg wet weight. Fish in some lakes in Uganda have much higher microcystin concentrations. In tropical climates, cyanobacterial blooms can occur year round and people are likely at greater risk because of constant, chronic exposure to microcystin in drinking water and fish (*34*). Cooking fish in the microwave or in boiling water decreases microcystin levels by 36–59% (*17*).

Microcystins also accumulate in crayfish (*40*) and blue crabs (*15*). Generally higher levels are detected in the intestine and hepatopancreas than in muscle tissue. Highest concentration of microcystin determined in blue crab muscle from Louisiana was 105 µg/kg wet weight. Shellfish (clams, oysters, mussels) are filter feeders and may consume cyanobacteria and concentrate cyanotoxins up to 107 times the level found in ambient water. High concentrations of microcystins may be present in the water of eutrophic freshwater rivers that empty into the ocean and nearby marine shellfish may become contaminated. This has been documented along the California coast where microcystin-poisoned sea otters have been observed (*27*).

There have been concerns that some foods that are not produced in aquatic environments might harbor microcystins. Providing drinking water containing toxigenic *Microcystis* (10^5 cells/ml) to beef cattle for a month did not cause liver dysfunction nor high levels of microcystin in liver tissue (*30*). Microcystins also did not accumulate to high levels in milk from dairy cows that consumed *Microcystis*-contaminated water (*31*). Microcystins in irrigation water do adhere to plant surfaces in the field, with highest concentrations found in the roots. If highly contaminated water must be used for irrigation, toxin levels in plants should be monitored (*14;28*).

Cyanobacteria ingested directly as food supplements may contain microcystins. Research published in 2000 reported that microcystins were detected in 85 of 87 commercial supplements tested and 63 samples contained >1 µg/g, the regulatory limit (*16*). Since then supplement producers have tested their products more

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frequently. Nevertheless, a 2008 paper reported that 94% of cyanobacterial supplements tested in China contained 2–163 ng microcystin/g (*19*). Some non-toxigenic cyanobacteria (*Nostoc commune* and *Spirulina plantensis*) are now cultivated in artificial ponds for use in supplements and these strains do not appear to have toxic effects in cell culture and animal tests (*45*). However, cyanobacteria do not generally grow in pure cultures, and some supplements labeled as containing *Spirulina* were found to also contain *Microcystis* and microcystin (*37*).

Microcystins are also present in the air near lakes with large cyanobacterial blooms and have been detected in persons using the lakes for recreation. Although ingestion is the main route of exposure, microcystins are much more readily absorbed from the nasal passages than from the gastrointestinal tract. Therefore, exposure to aerosolized microcystins may be a significant health risk to those participating in water sports or spending much time near lakes with cyanobacterial blooms (*2;43*).

Beta-methylamino-L-alanine (BMAA)

BMAA was first isolated from cycad seeds and evidence suggests that this neurotoxin is at least partially responsible for the amyotrophic lateral sclerosis-Parkinson's Disease (ALS-PD) complex that was endemic among indigenous people on Guam. Further research detected cyanobacteria of the genus *Nostoc* living symbiotically in specialized cycad roots, providing nitrogenous compounds for the cycad and synthesizing BMAA. Local people were chronically exposed to significant amounts of BMAA as they used cycad seeds for food and also consumed animals, such as fruit bats and pigs, that had fed on cycads and bioconcentrated BMAA. Analyses of brain tissues from ALS-PD victims demonstrated that they contained an average of 627 µg protein-bound BMAA/g tissue. Asymptomatic Guamanians had very low or undetectable levels of BMAA in their brains (*5*).

Unusual amino acids are not normally found in proteins but some are known to have significant physiological effects. BMAA is structurally similar to glutamic acid and can act on neuronal glutamate receptors, possibly causing overactivation, cell death, and neurodegeneration (*26*). BMAA has been associated with neurotoxic effects in cell cultures (*25*), birds (*4*), and rodents (*23;39*).

Lathyrism is another example of a neurological disease caused by consumption of an unusual amino acid, BOAA (β-N-oxalyl-amino-L-alanine), present in grass peas (*Lathyrus sativus*). BOAA also binds to glutamate receptors and induces other effects, resulting in paralysis in affected humans and animals (*41*).

More recently, research on BMAA has expanded beyond Guam. This amino acid is now known to be produced by other genera of cyanobacteria (*9*) and has been detected worldwide in lakes with algal blooms, including New Hampshire (*7*), Florida (*6*), The Netherlands (*13*), the Baltic Sea (*22*), and also in cyanobacterial crusts in the deserts of Qatar (*10*). BMAA has been detected in fish muscle tissue, shrimp, crabs, and cyanobacteria used as food and supplements (*6;22;29;38*).

Some studies have reported that brain tissue from patients who died of Alzheimer's Disease, amyotrophic lateral sclerosis, or Parkinson's Disease in Guam and Canada contained elevated levels of BMAA compared to brains of people that died from other causes (*29;32*). Exposure to BMAA may be one environmental factor responsible for development of sporadic cases of amyotrophic lateral sclerosis in Gulf War veterans and in persons living near lakes with regular cyanobacterial blooms (*7;10*). Since not all people with exposure to BMAA on Guam (and elsewhere) develop neurological disease, it is likely that certain people have an inherently greater susceptibility to this toxin. A recent review summarized and discussed data on BMAA as a cause of neurodegenerative diseases (*5*). More research is needed to determine the importance of BMAA and cyanobacteria to development of these diseases.

Paralytic Shellfish Poisoning (PSP) Toxins

Paralytic shellfish poisoning (PSP) occurs worldwide and is commonly associated with consumption of shellfish that have accumulated neurotoxins, the most potent of which are saxitoxins. Affected persons experience numbness, tingling, headaches, and difficulty breathing. Shellfish harvesting bans are usually implemented when toxin levels exceed 80 µg saxitoxin equivalents/100 g shellfish tissue. Saxitoxins have also been detected in some fin fish and in lobsters. Cooking does not inactivate these toxins (*12*).

Marine dinoflagellates (algae) of several genera are the major sources of PSP toxins. During algal blooms or "red tides," shellfish may consume enough algae to accumulate dangerous levels of PSP toxins. Certain freshwater and brackish water cyanobacteria also synthesize saxitoxins. For example, PSP toxins were detected in 10 of 39 German lakes, and cyanobacteria (*Aphanizomenon*) that produce PSP toxins were isolated from some of these lakes. These microbes may be the most important source of PSP toxins in fresh water (*3*).

Anatoxin-a

This toxin has a high acute toxicity when it is injected into rodents, causing death by respiratory failure and muscle paralysis. Although the toxin is readily absorbed after ingestion, it is also quickly degraded; therefore oral toxicity is low, with an LD_{50} in mice of

>5 mg/kg body weight (*14*). Anatoxin-a has been detected in some cyanobacterial dietary supplements labeled as Spirulina. Of 39 samples analyzed, anatoxin-a was detected in three supplements at concentrations of 2.5 to 33.0 µg/g. *Spirulina* is not known to produce anatoxin-a, indicating that these supplements may have contained other species of cyanobacteria (*Anabena*, *Oscillatoria*, or *Aphanizomenon*) or were mislabeled (*35*). Commercial Spirulina supplements generally recommend a dose of 3 g/day so it would appear that these levels would not be a health risk.

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FRI Briefings

Toxins from Algae/Cyanobacteria

first published May 1998
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***Pfiesteria piscicida* — Cell from Hell**

Pfiesteria spp. was identified last August 1997 as the cause of a major fish kill (30,000 fish) in Maryland's Pocomoke River and also affected fish in two other rivers on the eastern shore of Chesapeake Bay (*1*). In fact, since 1991 this one-celled dinoflagellate (a type of algae) has caused numerous major fish kills along the eastern coast of the USA, including a 1995 episode along the coast of North Carolina killing an estimated 15 million fish (*2,3*). Its current known range includes estuarine (brackish) and coastal waters as far north as Delaware and as far south and west as Alabama and Florida (*4*). A report from BBC News (*5*) indicates that *Pfiesteria* has also crossed the Atlantic to the British coast, perhaps in ballast tanks of cargo ships and tankers. This has raised concerns about potential massive fish kills along the European coast.

Pfiesteria has a complex life cycle with at least 24 distinct life stages. During winter and when food is scarce, cells rest as cysts in estuarine sediments until they chemically detect fresh fish excretions or secretions. The cysts then develop into motile, flagellated zoospores which secrete toxins inducing neurological symptoms such as lethargy and skin lesions—bleeding skin ulcers and a peeling away of the skin of their prey. The dinoflagellates then feed on the tissues of the dying fish. Concentrations of *Pfiesteria* zoospores at sites of fish kills range from 250 to 250,000/ml. When fish are not available, *Pfiesteria* will also consume some other algae, bacteria, and small animals. As environmental conditions become unfavorable, the cells synthesize a protective outer coating and sink to the sediment as cysts (*2,3,6*).

An influx of high concentrations of nutrients into coastal waters has been observed to stimulate the growth of noxious algae, including *Pfiesteria* (*7*). Following the rupture of a large North Carolina swine waste-holding lagoon in 1995, 25.8 million gallons of effluent flowed into a nearby coastal river. The immediate effect was depletion of oxygen and high levels of ammonia causing death of 4000 fish. As the effluent proceeded downstream, nutrient levels in the water increased dramatically and species of several nuisance algae, including *Pfiesteria*, bloomed and thousands more fish were killed or injured.

Although the nature of the *Pfiesteria* toxins has not been elucidated, there is direct evidence that they are toxic to humans. Both research scientists and coastal residents and fishermen have reported skin ulcers, short-term memory loss, confusion, prominent eye irritation, and respiratory distress following immersion in *Pfiesteria*-infested water or inhalation of aerosols from contaminated water (*6*). Most of the acute symptoms reversed with time if the persons avoided further exposure. However, some effects recurred following strenuous exercise. Therefore, all work with fish-killing cultures of *Pfiesteria* must now be conducted in biohazard level III containment systems in a limited-access facility (*3,4*).

Pfiesteria produces at least two toxins—one which is water-soluble and the other lipid-soluble. The hydrophilic compound is thought to be the neurotoxin (*4*). Preliminary in vitro studies demonstrated that nerve cells are particularly sensitive to one toxin as measured by decreased ATP levels and leakage of lactate dehydrogenase from the cells. Recent experiments with rats exposed to *Pfiesteria* demonstrated that they suffered a significant impairment in learning new tasks (8). The lipid-soluble toxin may be responsible for the skin lesions that are induced in exposed fish and humans.

Since affected fish are not very appetizing, foodborne illness due to consumption of these fish by humans does not appear likely at this time, nor would estuarine or ocean water containing the toxins likely be consumed. However, shellfish and crabs ingest other dinoflagellates that produce neurotoxins and accumulate enough saxitoxin, brevetoxin, or domoic acid to cause paralytic, amnesic, or neurotoxic shellfish poisoning in humans. Outbreaks of such foodborne disease have occurred in many coastal areas.

Preliminary studies indicate that shellfish also consume *Pfiesteria*. In some cases, adult shellfish did not appear to be affected; however, bay scallops ceased feeding after 15 minutes exposure to *Pfiesteria* zoospores (*4*). Therefore, there is a possibility that *Pfiesteria* could be filtered out by shellfish and the toxins accumulated to produce a new type of shellfish poisoning. It is important to establish whether or not *Pfiesteria* causes foodborne illness via fish or shellfish, because there is a public perception that it may do so. Following the Chesapeake Bay outbreak last year, it was estimated that the "*Pfiesteria* panic" caused a reduction in total sales from $253 million to $210 million for businesses that specialize in seafood. Seafood sales in restaurants and stores dropped dramatically in September/October although they later recovered (*4*).

Microcystins

These toxins, produced by cyanobacteria (sometimes called blue-green algae), were also in the news recently as they caused acute liver failure in more than 100 Brazilian hemodialysis patients (*9*). Symptoms were first manifested Feb. 17–20, 1996, and within 7 months at least 50 patients died of the acute hepatotoxic effects of these toxins. Microcystin concentrations in liver samples from the victims were comparable to those found in laboratory animals given lethal doses of this toxin. An investigation of the outbreak revealed that visibly turbid reservoir water was supplied to the center by truck. Despite some water treatment at the dialysis facility (filtration, carbon adsorption, and deionization), the patients were exposed to high levels of microcystins probably because the filters and carbon adsorption tank had not been changed for at least 3 months. No chemical treatment (chlorination) step was used. Since the toxins were introduced directly into the bloodstream, these patients were more severely exposed than persons who might have drunk the contaminated water.

In most cases, water treatment systems should be adequate to remove cyanobacteria by coagulation and filtration and microcystins can be removed by charcoal filters and degraded by chlorination (*10*). However, we all remember the *Cryptosporidium* outbreak in Milwaukee a few years ago when lack of proper maintenance of a water treatment system allowed this protozoan to pass through the filtration system and into the city's drinking water. (See previous FRI update on *Cryptosporidium*.) Tests with domestic water filters indicated that they could remove some cyanobacteria and microcystins but none of the models tested removed all the cells or toxins (*11*).

Cyanobacterial toxins have also been reported to cause epidemic gastroenteritis in humans in Brazil (*9*) and death in cattle and other animals who drank contaminated water. Poisoning of cattle and other domestic animals often occurred when their drinking water source was a eutrophic pond receiving significant agricultural runoff (*12*). Occasionally, toxin-producing cyanobacteria are reported from remote oligotrophic lakes which are low in plant nutrients and have high oxygen levels (*13*).

Although there have been no reported incidents of poisoning with cyanobacterial toxins present in foods, shellfish can filter cyanobacteria from water and could accumulate these toxins. Saltwater mussels (*Mytilus edulis*) fed *Microcystis* accumulated microcystins which persisted for several days after transfer of the mussels to clean water (*14*). Fresh fruits and vegetables washed in contaminated water could also acquire these toxins. Dietary supplements containing blue-green algae may contain microcystins (pers. comm., Dr. F. S. Chu) and are another potential source of human exposure

to cyanobacterial toxins.

Cyanobacteria are common in fresh water throughout the world and are known to produce several types of toxins: **microcystins** which are heat-stable, cyclic heptapeptide hepatotoxins and also promote tumor growth and inhibit protein phosphatases; **anatoxin**, a neurotoxin, which inhibits acetylcholinesterase; and **paralytic shellfish poisoning (PSP) toxins**, such as saxitoxin, which are also neurotoxic. *Lyngbya wollei*, commonly found in some lakes and reservoirs in the southeastern USA, and one strain of *Aphanizomenon flos-aquae* also produce potent neurotoxins, related to PSP toxins (*15*). Toxin levels produced by *Anabaena* spp. were much greater in older cultures and in cultures supplemented with phosphorus (*16*). Therefore, a reduction of phosphorus loads in ponds and reservoirs might aid in preventing toxic cyanobacterial blooms.

Algae are important as the base of nearly all aquatic food chains but some varieties have become a nuisance fouling our lakes. Unicellular eukaryotic algae and prokaryotic blue-green algae are widespread in various bodies of water throughout the world. But they are often overlooked until their populations explode (bloom) and/or they produce toxins affecting humans or domestic animals. Several investigators have reported that such incidents are becoming more common and attribute this to addition of more nutrients to water: runoff from agricultural lands or concentrated farming operations or from nearby homeowners adding excess fertilizer to their lawns. An issue of the journal *Limnology and Oceanography* (*17*) last summer was devoted to the ecology of harmful algal blooms (*2*).

Since some of these organisms have the potential to cause significant outbreaks of illness in humans and animals, we must be vigilant in maintaining our water purification systems and also be aware of the quality of the water used by our domestic animals. The U.K. and the State of Oregon have set limits of 1 ppm for microcystins in water and dietary supplements. The U.S. government has developed and published a National Harmful Algal Bloom Research and Monitoring Strategy which will initially focus on *Pfiesteria* outbreaks (*18*). Many experts believe that we will experience additional outbreaks of illness related to blooms of cyanobacteria or dinoflagellates until we understand how to control the environmental factors that limit their growth.

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Last modified: 10 June 1998

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