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Project Summary

A Status Report on Planktonic Cyanobacteria (Blue-Green Algae) and their Toxins

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While several groups of algae can cause dense waterblooms, blue-green algae are the most common offenders. Blue-green algae — also called cyanobacteria — are minute, single-celled microorganisms that lack a distinct, membrane-bound nucleus but, because they contain chlorophyll, can photosynthesize.

Surface blooms or scums of cyanobacteria commonly occur during the warm, windless days of late summer and fall when water can stagnate and when there are sufficient concentrations of such nutrients as nitrogen and phosphorus. Nutrient levels that contribute to water-bloom formation can result from runoff of fertilizers or livestock or human wastes.

While cyanobacterial blooms can affect the water's taste, odor, and appearance, they also pose a more serious problem. Most, if not all, of the common bloom-forming cyanobacteria can produce potent biotoxins. These toxins, formed at all stages of the organisms' growth, generally remain inside the cell until age or stress causes their release into the surrounding water. The main toxic genera include filamentous *Anabaena, Aphanizomenon, Nodularia, Nostoc, Oscillatoria,* and unicellular *Microcystis.* More than one species within these genera can be toxic, and all toxic species can form waterblooms.

Toxic waterblooms can take place in many eutrophic (nutrient rich) to hypereutrophic lakes and ponds at temperate latitudes worldwide. They are responsible for sporadic but recurrent episodes of illness and death among wild and domestic animals. Algal toxins have also been implicated in human poisonings from certain municipal and recreational water supplies. This implication is important for public health officials and water management personnel who need to be aware of the significance of the threat to health from these water-based toxins. The full report provides a comprehensive assessment of toxins from cyanobacteria and a directory to the literature published on this subject.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Toxic waterblooms of cyanobacteria can be found in many eutrophic to hypereutrophic lakes, ponds and rivers throughout the world (Table 1). The primary toxicoses that result from ingesting toxic cyanobacteria or their toxins include acute liver toxicosis, rapid neurotoxicosis, and gastrointestinal disturbances. Most cases, known from wild and domestic animal poisonings, involve the first two types. They result from ingesting lethal or sublethal numbers of toxic cells from a toxic waterbloom.

Most cyanobacterial poisonings result in hepatotoxicosis. The algal hepatotoxins are a related family of low-molecular-weight cyclic hepta- and pentapeptides called

Table 1. Known Occurrences of Toxic Cyanobacteria in Fresh or Marine Water

Argentina
Australia
Chile
Bangladesh
Bermuda
Brazil

Canada
- Alberta
- British Columbia
- Manitoba
- Ontario
- Saskatchewan

Europe
- Czechoslovakia
- Denmark
- Finland
- France
- Germany
- Greece
- Hungary
- Italy
- Netherlands
- Norway
- Poland
- Portugal
- Russia
- Sweden
- Ukraine
- United Kingdom

India
Israel
Japan
New Zealand
Okinawa (marine only)
Peoples Republic of China
South Africa
Thailand

U.S.A.
- California
- Colorado
- Florida
- Hawaii (marine only)
- Idaho
- Illinois
- Indiana
- Iowa
- Michigan
- Minnesota
- Mississippi
- Montana
- Nebraska
- Nevada
- New Hampshire
- New Mexico
- New York
- North Dakota
- Ohio
- Oklahoma
- Oregon
- Pennsylvania
- South Dakota
- Texas
- Washington
- Wisconsin
- Wyoming

microcystins and nodularins (Table 2). Of the peptide-toxin producing genera, *Microcystis* is the main offender worldwide. Animals affected by these hepatotoxins may display weakness, anorexia, and pallor of the extremities and mucous membranes. Since all animals in a herd or flock usually drink from the same water supply, most members will be affected within the same time. Death occurs after liver cell architecture loss, which leads to destruction of the parenchymal cells and sinusoids of the liver. This causes lethal intrahepatic hemorrhage within minutes to hours or hepatic insufficiency within several hours to a few days.

Cyanobacterial neurotoxicosis results from ingestion of toxic *Anabaena, Aphanizomenon* or *Oscillatoria*. While these genera can produce peptide hepatotoxins as well as neurotoxins, the neurotoxins act more rapidly and dominate the field and clinical syndrome. The neurotoxins comprise two groups: anatoxins and saxitoxins (Table 2). There are two known anatoxins: anatoxin-a and anatoxin-a(s). Anatoxin-a is a bicyclic secondary amine that blocks the postsynaptic transmission of nerve impulses by depolarizing (stimulating) the neurons that receive the signal. The mechanism is similar to what the natural chemical transmitter, acetylcholine, does in stimulating muscle contraction. However, unlike acetylcholine, anatoxin-a is not physiologically regulated and causes continuous depolarization that leads to muscle fatigue and paralysis. Anatoxin-a(s), is an organophosphate (OP) cholinesterase inhibitor that acts like an OP pesticide overdose. Here, the toxin inhibits the natural enzyme, acetylcholinesterase, from recycling the transmitter, acetylcholine. Both toxins produce symptoms that include muscle twitching and contraction, reduced movement, gasping respiration, cyanosis (bluish color from poorly oxygenated blood), convulsions, and death. Neuromuscular blockage of the muscles used in breathing is the most likely cause of death.

Although both toxins are respiratory neurotoxins, anatoxin-a produces a rigid neck contracture in birds as a result of its depolarizing activity, while anatoxin-a(s) causes intense salivation and mucous nasal discharge as a result of its anticholinesterase activity. Investigators can use these two very different signs of poisoning in avian species to differentiate the two toxins, either in field poisonings or in laboratory assays.

Aphanizomenon has been shown in some cases to produce the potent sodium channel neuromuscular blocking agents, saxitoxin and neosaxitoxin. These two neurotoxins are better known from being produced by marine dinoflagellate algae responsible for the red tide poisoning phenomenon, paralytic shellfish poisoning (PSP).

Potential for Human Poisoning

All cyanotoxins could cause death or illnesses in humans as well as in wild and domestic animals. Yet many officials remain unconvinced of the need to monitor or regulate these toxins in municipal or recreational water supplies. The skepticism seems to arise from the fact that, despite the presence of cyanobacteria in many bodies of water there are no confirmed cases of human death or illness from their toxins.

Several factors, which probably act in combination, may explain the lack of reported human toxicity:

- Mollusks that concentrate toxins the way shellfish concentrate marine PSP toxins are uncommon in freshwater. Where they do exist, as in Europe, people tend not to eat freshwater shellfish, except locally.
- Cyanotoxins induce lethal toxicity at a very low concentration, but they have a steep dose-response curve. In other words, animals must swallow a lethal or nearly lethal dose before signs of poisoning are observable. Such high concentrations of toxin occur only when waterblooms accumulate on the water's surface, especially on the downwind shore. While this is certainly the most dangerous for watering animals, humans often find the waterblooms' sight and smell repulsive.
- Most water supplies in North America and Europe don't support high concentrations of toxic cyanobacteria year round, largely because of better water quality management and colder winters. While toxic waterblooms do occur in some drinking water supplies, filtration and dilution reduce lev-

Table 2. Comparison of Toxicities of Some Biological Toxins

Toxin	*Source*	*Common Name*	*Lethal Dose** (LD_{50})
Botulinum Toxin	*Clostridium botulinum*	(Bacterium)	0.00003
Tetanus Toxin	*Clostridium tetani*	(Bacterium)	0.0001
Ricin	*Ricinus communis*	(Castor Bean Plant)	0.02
Diphtheria Toxin	*Corynebacterium diphtheriae*	(Bacterium)	0.3
Kokoi Toxin	*Phyllobates bicolor*	(Poison Arrow Frog)	2.7
Tetrodotoxin	*Arothron meleagris*	(Puffer fish)	8
Saxitoxin	*Aphanizomenon flos-aquae* & *Alexandrium sp.*	**(Cyanobacteria)** (Dinoflagellate)	9
Cobra Toxin	*Naja naja*	(Cobra Snake)	20
Anatoxin-a(s)	*Anabaena flos-aquae*	**(Cyanobacteria)**	20
Nodularin	*Nodularia spumigena*	**(Cyanobacteria)**	50
Microcystin	*Microcystis, Anabaena, Oscillatoria, Nostoc*	**(Cyanobacteria)**	50-500
Anatoxin-a	*Anabaena flos-aquae*	**(Cyanobacteria)**	200
Amatoxin	*Amanita sp.*	(Fungus)	200-500
Curare	*Chrondodendron tomentosum*	(Brazilian Poison Arrow Plant)	500
Strychnine	*Strychnos nox-vomica*	(Plant)	500
Muscarin	*Amanita muscaria*	(Fungus)	1100
Phallotoxin	*Amanita sp.*	(Fungus)	1500-2000
Sodium Cyanide			10000

*The acute LD_{50} in µg per kg bodyweight: intra-peritoneal injection; some with mice, some with rats.

els in the finished water below those that cause acute toxicosis. Further, without sensitive detection methods it's difficult to determine how much is in the finished drinking water, and without a clear understanding of the toxins' mechanisms of action it's difficult to determine whether they are causing subacute or chronic toxicosis in humans.

Effects of Low-Level Exposure

The most likely threat to human health from cyanobacterial toxins is subacute and chronic toxicity. We know from research over the past 20 years what the most likely mode of death will be from a lethal dose. What we don't know is the mechanism of action for a nonlethal dose, whether from a single exposure or from chronic exposure to a drinking water supply containing a persistent toxic waterbloom. There is indirect evidence that low-level concentrations of peptide hepatotoxins in drinking water affect the liver and intestine. Recent research shows that the peptide toxins are potent inhibitors of protein phosphatases type 1 and 2A. This means that they are tumor promoters similar in action to okadaic acid, the causative agent of diarrhetic shellfish

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poisoning. Research currently underway at the National Cancer Research Institute in Tokyo shows these peptide toxins can produce liver tumors in laboratory rodents. Thus, the continuous low-dose exposure that would occur during a summertime waterbloom could possibly promote liver tumor formation in humans.

Recommendations for Research and Development

A. Continue efforts to develop predictive models to quantify the formation of cyanobacterial blooms. These models should be developed with thought toward their use for devising management plans for various water bodies.

B. Further research to develop measures to control eutrophication and minimize development of cyanobacteria waterblooms and scums.

C. Support development of sensitive, rapid and accurate methods for the detection of cyanotoxins.

D. Support efforts to adopt standard procedures for characterization of the cyanotoxins that would in turn support efforts to make toxin standards available for research.

E. Support research leading to an understanding of the transport, fate, and ecological role of cyanotoxins in aquatic environments.

F. Support studies of the physiological and genetic mechanisms involved in toxin production. Cyanobacteria can be genetically manipulated and studied with many of the same techniques available to study molecular and cellular genetics of other prokaryotes.

G. Support studies on the taxonomy and classification of cyanobacteria. This information is critical to effective communication about toxic cyanobacteria and their toxins.

H. Develop USEPA support to carry out several aspects of the work on cyanobacterial toxins. These include:

1. Educate and advise Federal, State and local public health workers and the general public on toxic cyanobacteria.
2. Link work on cyanotoxins with appropriate authorities in other parts of the world so that information can be exchanged and collaborative research projects can be developed and supported.

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The complete report, entitled "A Status Report on Planktonic Cyanobacteria (Blue-Green Algae) and Their Toxins," (Order No. PB92- 206 259/AS; Cost: $25.00; subject to change) will be available only from:
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