

Cyanotoxins and the health of domestic animals and humans

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Outline

- 1. Cyanotoxins: chemical structure and their effects on mammals**
- 2. Case examples of impacts on domestic animals and humans**
- 3. Modes of exposure**
- 4. Puzzle: search for a link between cyanobacteria and neurological disease in New Hampshire**

| Toxin group ¹ | Primary target organ in mammals | Cyanobacterial genera ² |
|----------------------------------|--|---|
| <i>Cyclic peptides</i> | | |
| Microcystins | Liver | <i>Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis</i> |
| Nodularin | Liver | <i>Nodularia</i> |
| <i>Alkaloids</i> | | |
| Anatoxin-a | Nerve synapse | <i>Anabaena, Planktothrix (Oscillatoria), Aphanizomenon</i> |
| Anatoxin-a(S) | Nerve synapse | <i>Anabaena</i> |
| Aplysiatoxins | Skin | <i>Lyngbya, Schizothrix, Planktothrix (Oscillatoria)</i> |
| Cylindrospermopsins | Liver ³ | <i>Cylindrospermopsis, Aphanizomenon, Umezakia</i> |
| Lyngbyatoxin-a | Skin, gastro-intestinal tract | <i>Lyngbya</i> |
| Saxitoxins | Nerve axons | <i>Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis</i> |
| <i>Lipopolysaccharides (LPS)</i> | Potential irritant; affects any exposed tissue | All |

Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management

Edited by Ingrid Chorus and Jamie Bartram

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Major known cyanotoxins and their health-related effects

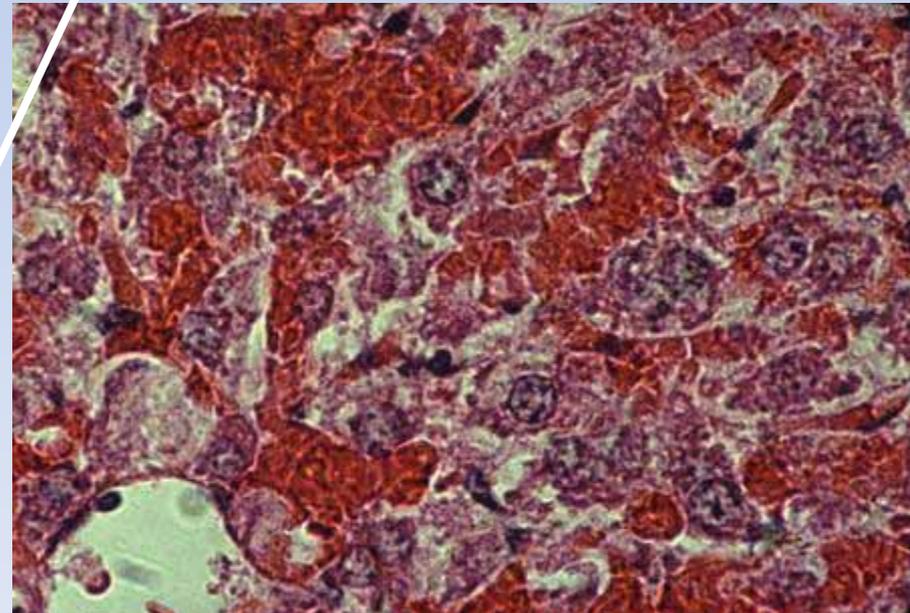
| Toxin | Target Organ | Symptoms of Exposure |
|--|----------------|---|
| Microcystins/Nodularins | Liver | Acute: hepatocyte apoptosis (live cell death), death in days Chronic: linked to liver & colon cancer, tumor growth |
| Anatoxin a/Anatoxin a(s) | Nerve synapses | Convulsions, rapid death; Often associated with deaths of dogs and birds |
| Saxitoxin/Neurotoxin | Nerve axons | Numbness, trembling, paralysis, death by respiratory arrest; interference with neurodevelopment in fish |
| Cylindrospermopsins | Liver | Mainly hepatic and renal effects in humans; poorly studied |
| β - <i>N</i> -methylamino-L-alanine (BMAA) | Nerve | Neurotoxic effect appears to be release of excess calcium in the neurons; may be linked to neurological diseases |

Following Microcystin Injection

Normal Mouse Liver



Within 2-4 hours liver capillaries break down, flooding the liver with blood



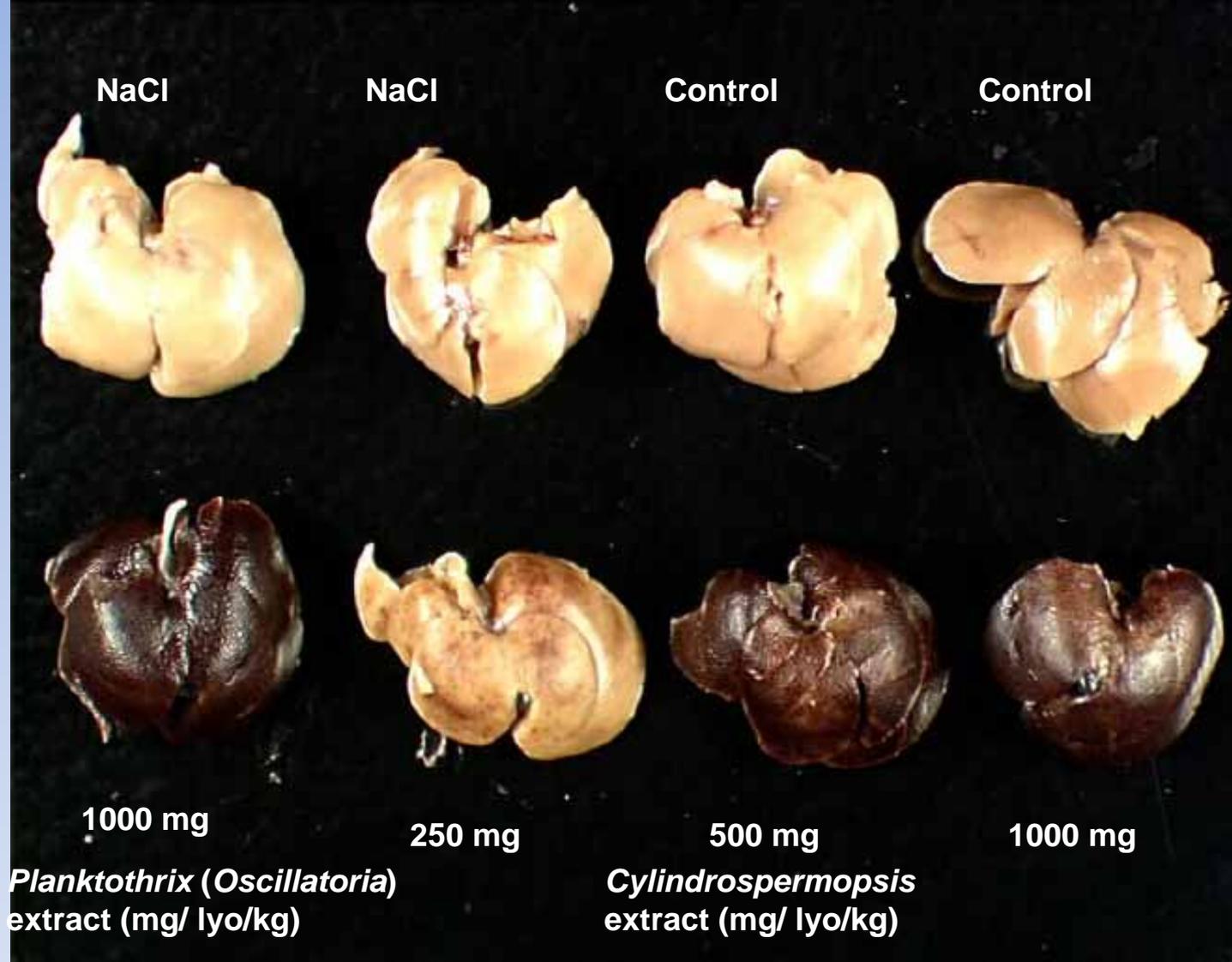
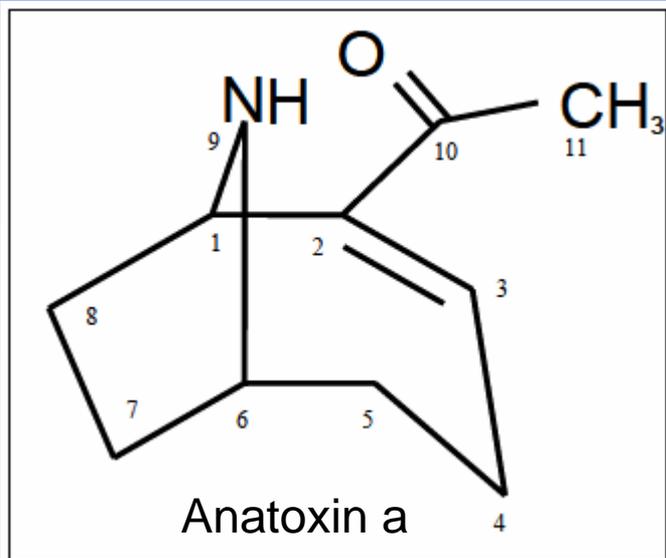


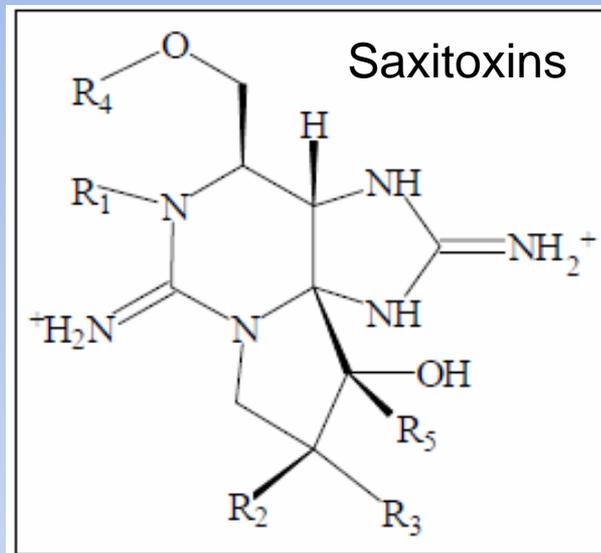
Figure 2. Macroscopic pathology of fixed livers from Male Swiss Albino mice (IOPS OF1 strain) treated with toxic extracts (unpublished results from Mathilde Harvey, AFSSA Paris). Top row from left to right: two mouse livers injected with 0.9% NaCl and two non-perfused mice livers as controls. Bottom row: liver of mouse injected with an extract (20 mg lyophilised sample/mL, 0.9% NaCl used as extraction solvent, i.e. 1000 mg lyo/kg of body weight) of a field sample of *Planktothrix agardhii* (producing microcystins) and with culture extracts (5, 10 and 20 mg lyophilised sample/mL, 0.9% NaCl, i.e. 250, 500 and 1000 mg lyo/kg of body weight) of *Cyindrospermopsis raciborskii* (producing cylindrospermopsin).

Neurotoxins



Nicotinic acetylcholine receptor agonists . Residence of these toxins at post-synaptic cholinergic receptors results in nerve depolarisation (Swanson et al. 1990; Huby et al. 1991; Swanson et al. 1991; Wonnacott et al.1991).

Typical symptoms in mice are loss of muscle coordination, gasping, convulsions and death within minutes from respiratory arrest (Carmichael et al. 1979).



Paralytic shellfish poisons (PSP). These toxins are potent voltage-gated sodium channel antagonists, causing numbness, paralysis and death by respiratory arrest..

Accumulate in shellfish.

Cyanotoxins: Case Examples

Dogs = “Freshwater Coal Mine Canaries”



WHY?

Dogs love to drink
ANYWHERE





Blooms typically have a patchy distribution

Microcystis Bloom - East bank of the St. Johns River - Mandarin - 08.19.05 - 2:42pm

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The Lake Champlain

September 1999

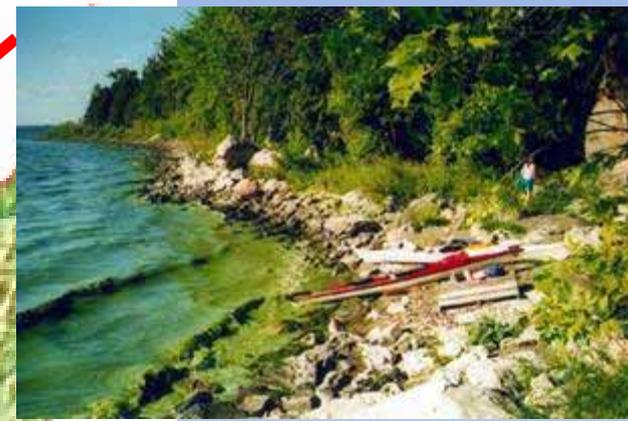
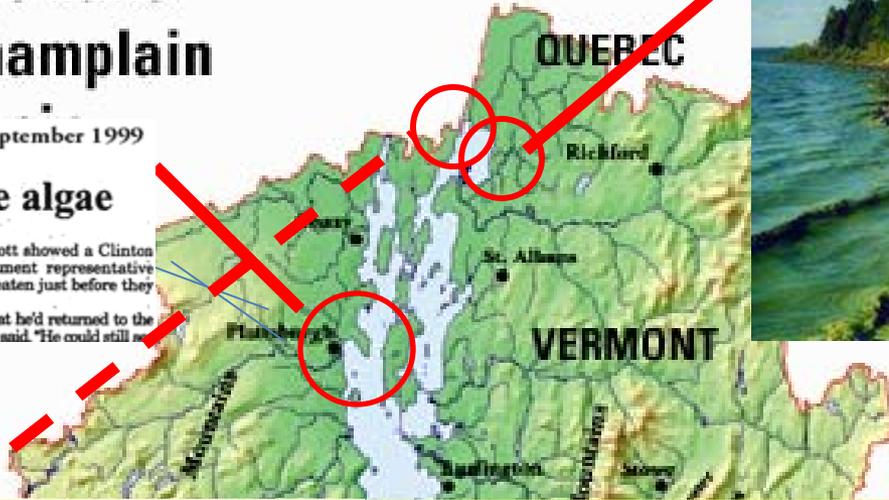
Dog deaths laid to poison lake algae

By SUZANNE MOORE
and MARCIA LANHEAR
Staff Writers

PLATTSBURGH — John Linscott had walked his dogs at Point au Roche State Park more times than he could remember.

... makes me crazy." Two days later, Linscott showed a Clinton County Health Department representative the plant his dogs had eaten just before they got sick.

There was no doubt that he'd returned to the fateful spot, Mrs. Linscott said. "He could still see



Summer 2002

Blue-Green Algae
in Bay
Dog

Probable cause: Anatoxin a

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Algae blooms a growing problem
Don't touch the water, cottage country warned

Graeme Hamilton
National Post

VENISE-EN-QUEBEC, Que. - The campground was immaculate and the beach freshly raked, but on a perfect



Deaths
Burlington - The Vermont Department of Health has received word that two dogs died after ingesting water from Lake Champlain in the Missisquoi Bay area. One dog had been swimming in the Canadian part of the lake, the other near Highgate Springs. Both deaths occurred in the last two weeks.

Box 4.5 Examples of health effects due to recreational exposure

1959 Saskatchewan, Canada. In spite of recreational use warnings and deaths in livestock, people swam in a lake infested with cyanobacteria. Thirteen persons became ill (headaches, nausea, muscular pains, painful diarrhoea). In the excreta of one patient (a medical doctor who had accidentally ingested 300 ml of water), numerous cells of *Microcystis* spp. and some trichomes of *Anabaena circinalis* were identified (Dillenberg and Dehnel, 1960).

1989 United Kingdom. Ten of 20 army recruits showed symptoms indicating intoxication (e.g. vomiting, diarrhoea, central abdominal pain, blistering of the lips, sore throats) after swimming and canoe training in water with a dense bloom of *Microcystis* spp. Two of the recruits developed severe pneumonia attributed to the aspiration of a *Microcystis* toxin and needed hospitalisation and intensive care (Turner *et al.*, 1990). The severity of illness appeared to be related to the swimming skills and amount of water ingested.

1995 Australia. Epidemiological evidence of adverse health effects after recreational water contact was established in a prospective study involving 852 participants. Results showed an elevated incidence of diarrhoea, vomiting, flu symptoms, skin rashes, mouth ulcers, fevers, eye or ear irritations within seven days following exposure (Pilotto *et al.*, 1997). Symptoms increased significantly with duration of water contact and cell density of cyanobacteria.

Box 4.3 Palm Island Mystery Disease

In 1979, there was a major outbreak of hepato-enteritis among the children of an Aboriginal community living on a tropical island off the coast of Queensland, Australia. Altogether 140 children and 10 adults required treatment, which was provided by the local hospital for less severe cases and by the regional hospital on the mainland for severe cases possibly requiring intensive care. Diagnostic information included a detailed clinical examination showing malaise, anorexia, vomiting, headache, painful liver enlargement, initial constipation followed by bloody diarrhoea and varying levels of severity of dehydration. Urine analysis showed electrolyte loss together with glucose, ketones, protein and blood in the urine, demonstrating extensive kidney damage. This was the major life-threatening element of the poisoning. Blood analysis showed elevated serum liver enzymes in some children, indicating liver damage. Sixty-nine percent of patients required intravenous therapy and in the more severe cases the individuals went into hypovolaemic/acidotic shock. After appropriate treatment all the patients recovered (Byth, 1980).

Examination of faecal samples and foods eliminated a range of infectious organisms and toxins as possible causes for the outbreak, hence the name "Palm Island Mystery Disease". The affected population, however, all received their drinking water supply from one source, Solomon Dam. Families on alternative water supplies on the island were not affected by the disease.

Prior to the outbreak of the illness an algal bloom occurred in Solomon Dam. The bloom discoloured the water and gave it a disagreeable odour and taste. When the bloom became dense, the dam reservoir was treated with 1 ppm of copper sulphate (Bourke *et al.*, 1983).

Clinical injury among consumers on that water supply was reported the following week.

The organisms from the dam were cultured and administered to mice, following which the mice slowly (over several days) developed widespread tissue injury involving the gastrointestinal tract, the kidney and liver (Hawkins *et al.*, 1985). The widespread tissue damage and delayed effects are quite different to those following *Microcystis aeruginosa* administration (Falconer *et al.*, 1981; see section 4.2.1).

Subsequent monitoring of the algal blooms in the dam identified the cyano-bacterium *Cylindrospermopsis raciborskii* as the cause of the blooms, with seasonal cell concentrations of up to 300,000 cells per ml of water. This organism does not form scums, and has the highest cell concentrations well below the water surface. In order to reduce bloom formation, the responsible

Box 4.4 Outbreak of severe hepatitis following haemodialysis in Caruaru, Brazil

In February 1996, an outbreak of severe hepatitis occurred at a Brazilian haemodialysis centre in Caruaru, Brazil, located 134 km from Recife, the state capital of Pernambuco. At this clinic 117 of 136 patients (86 per cent) experienced visual disturbances, nausea, vomiting, muscle weakness and painful hepatomegaly, following routine haemodialysis treatment. Subsequently, 100 patients developed acute liver failure and 50 of these died. As of October 1997, 49 of the deaths could be attributed to a common syndrome now called "Caruaru Syndrome". This syndrome includes:

- *Symptoms.* Painful huge hepatomegaly, jaundice and a bleeding diathesis manifested by ecchymosis, epistaxis and metrorrhagia.
- *Laboratory picture.* Elevated transaminases, variable hyperbilirubinemia, prolonged prothrombin time and severe hypertriglyceridemia.
- *Histopathology.* Light microscopy - disruption of liver plates, liver cell deformity, necrosis, apoptosis, cholestasis, cytoplasmic vacuolisation, mixed leukocyte infiltration and multinucleated hepatocytes; electron microscopy - intracellular oedema, mitochondrial changes, rough and smooth endoplasmic reticulum injuries, lipid vacuoles and residual bodies.

Symptoms indicate microcystin poisoning

Anatoxin-a

Case study: dog deaths in France, 2003 (Gugger et al. 2005).

Two dogs died in separate incidents in September 2003 shortly after drinking from the shore of a river in the Jura region. Clinical signs were vomiting, hind limb paresis and respiratory failure preceding death. The smaller dog (2.5kg) sickened and died shortly after emerging from the water, whereas the larger dog (25kg) had a delayed onset of signs and died within five hours. Stomach contents, intestinal contents and liver were sampled as well as water column and benthic biofilm from the river; stomach contents and field samples were examined for phytoplankton identification. An ini-

Microcystins

Case study: duck deaths in Japan, 1995 (Matsunaga et al. 1999)

Nodularin

Case study: dog death in South Africa, 1994 (Harding et al. 1995).

Cylindrospermopsin

Case study: cattle deaths in Australia, 2001 (Shaw et al. 2004).

Saxitoxins

Case study: sheep deaths in Australia, 1994 (Negri et al. 1995).

Thirteen ewes and one ram died next to or within 150m of a farm dam. Observed signs were trembling, recumbency and crawling. The ram, which

Anatoxin a & Microcystins

Case study: waterbird deaths in Denmark, 1993 (Henriksen et al. 1997, Onodera et al. 1997).

Cyanobacteria-associated animal deaths have been reported at Lake Knudsø since 1981. In 1993, two grebes and a coot that died when a cyanobacterial bloom was evident were collected and frozen. Stomach contents were examined microscopically to identify cyanobacteria; *Anabaena lemmermannii* were found in all three birds. This material was lyophilised for

Unusual Cases



A Wave of Momentum for Toxic Algae Study

Author(s): Carla Burgess

Source: *Environmental Health Perspectives*, Vol. 109, No. 4 (Apr., 2001), pp. A160-A161

Did humans create Cyanoblooms?

Braun and Pfeiffer (2002) present their hypothesis that at Neumark–Nord in Germany, a Pleistocene (1.8 million – 11,000 years ago) lake assemblage of >70 deer, as well as forest elephant, rhinoceros, auroch (ox) and cave lion skeletons may represent a cyanotoxin–related mass mortality event.

“Unusual” animal deaths and cyanobacteria

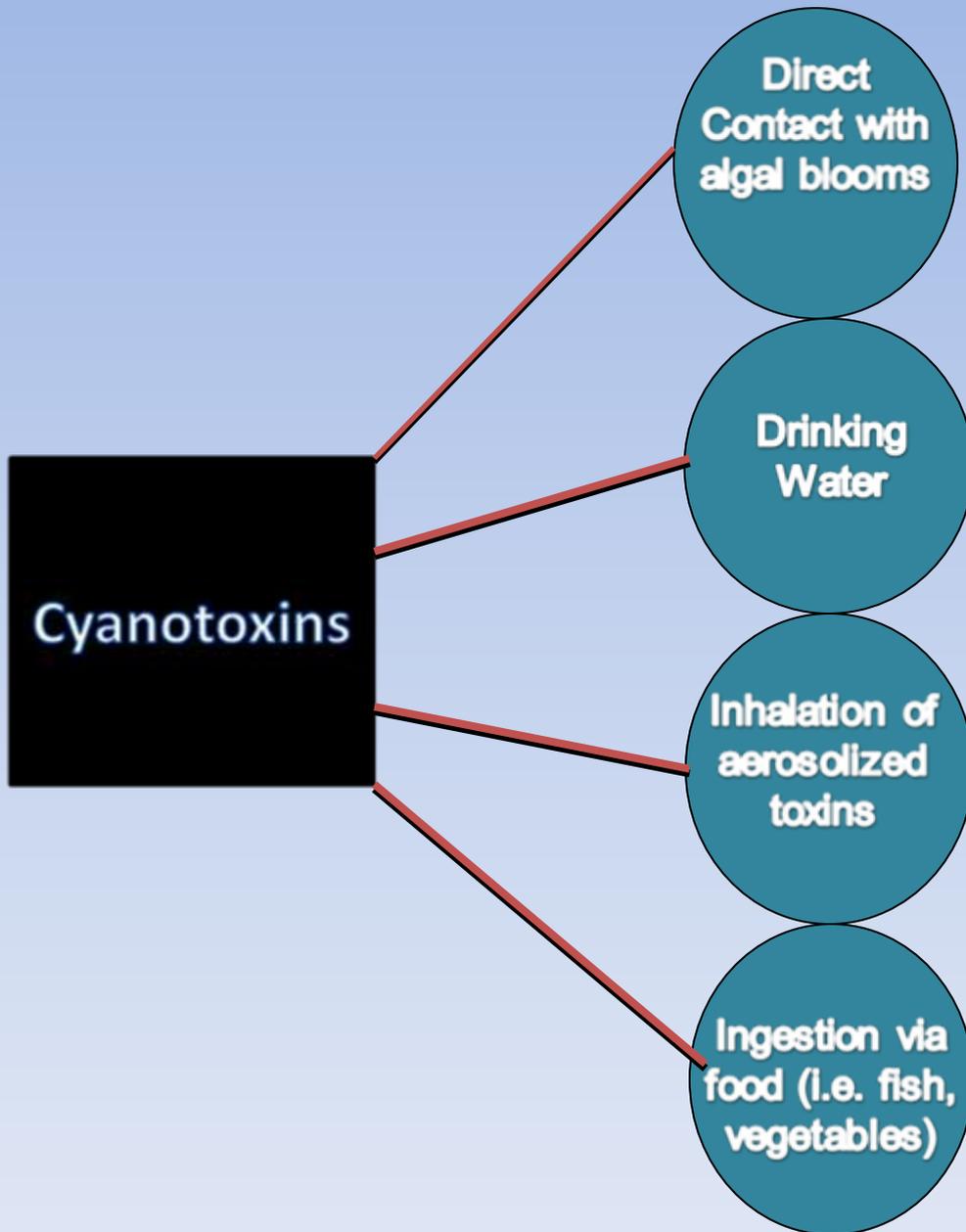
The previous discussion has concentrated on cyanotoxin-related deaths in common domestic, stock and wild animals. Some other animals reportedly poisoned by cyanobacteria are:

- **Flamingos:** Cyanobacteria-related mortalities have been reported in three flamingo species, both wild and captive (see summary by Codd et al. (2003)). Lesser Flamingos in Kenyan soda lakes have been the subject of research interest, with four microcystin congeners and anatoxin-a found in cyanobacterial mats and stomach contents of dead birds at Lake Bogoria. Microcystins and anatoxin-a were also detected in faecal pellets collected from lake shorelines (Krienitz et al. 2003). The same cyanotoxins have been found in feathers taken from poisoned flamingos, with a dietary origin most likely (Metcalf et al. 2006). Mass die-offs of tens of thousands of birds have been reported in these crater lakes, with implications for management and regional and national economies, as flamingos are a significant tourist attraction (Krienitz et al. 2003, Ndetei and Muhandiki 2005). Isolated strains of *Arthrospira fusiformis* from two crater lakes have been shown to produce both microcystin-YR and anatoxin-a, while an *A. fusiformis* strain from a third lake produces anatoxin-a (Ballot et al. 2004, Ballot et al. 2005). Similar Lesser Flamingo poisonings have been reported from alkaline lakes in Tanzania, with toxic *A. fusiformis* implicated (Lugomela et al. 2006). The implications of these findings are significant for several reasons, as *Arthrospira* sp. (also known as *Spirulina*) are the principal food source of Lesser Flamingos, and *Spirulina* spp. are used as a dietary supplement by humans and as a feed additive for livestock. There is a significant body of literature that suggests that consumption of *Spirulina* is not harmful (Ciferri 1983, Belay et al. 1993, Hayashi et al. 1994, Qureshi et al. 1996, Salazar et al. 1998, Abdulquader et al. 2000, Al-Batshan et al. 2001), though presumably these studies refer to non-toxic strains of *Arthrospira* used for both commercial mass production and from wild harvesting. Investigations to determine the relative contribution of *A. fusiformis* to the production of cyanotoxins in Kenyan soda lakes would be of great interest. Some of these lakes are periodically dominated by more well-known toxigenic cyanobacteria such as *Anabaena* and *Microcystis* spp. (Ndetei and Muhandiki 2005),

so it will be important to estimate the production capacity of cyanotoxins by various cyanobacteria in field situations when harmful levels of cyanotoxins are present.

- **Insectivorous bats:** Staff at a campground in Alberta, Canada, in the summer of 1985, counted 500 dead bats and estimated over 1,000 deaths on the leeward side of a lake. At least 24 dead mallards were also reported. The lake area was covered with a “thick white scum” that had a blue-green sheen. Examined animals were covered with a green slime; necropsy did not reveal any abnormalities. An alkaloid was extracted from the material covering the carcasses. This alkaloid was identified by GC/MS and found to be anatoxin-a (known at the time as Very Fast Death Factor) (Pybus et al. 1986).
- **Rhinoceros:** Four white rhinoceroses were introduced to a South African game reserve in May, 1979. Two months later, two of them were found dead but were unable to be examined. Approximately one week later, another rhino was found dead after being seen to be active the previous day. Macroscopic and microscopic findings were typical of acute hepatotoxicity: hepatomegaly, ascitic fluid, coagulopathy seen in various tissues, severe hepatic necrosis and loss of hepatic architecture. At the time of death, a severe bloom of *M. aeruginosa* covered the park dam, with a surface scum of 4–12cm (Soll and Williams 1985).
- **Honeybees:** In the summer of 1971, “almost total” mortality of bees from 84 hives was associated with the insects watering on the leeward edge of a lake in New South Wales, Australia. That area of the lake was affected by a windborne scum of *A. circinalis*; an apiary on the windward shore was unaffected (May and McBarron 1973).

Potential Pathways of Exposure to Cyanotoxins



Other means?

- Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands





Colonies of *Nostoc commune*: a delicacy in the Andes of Peru

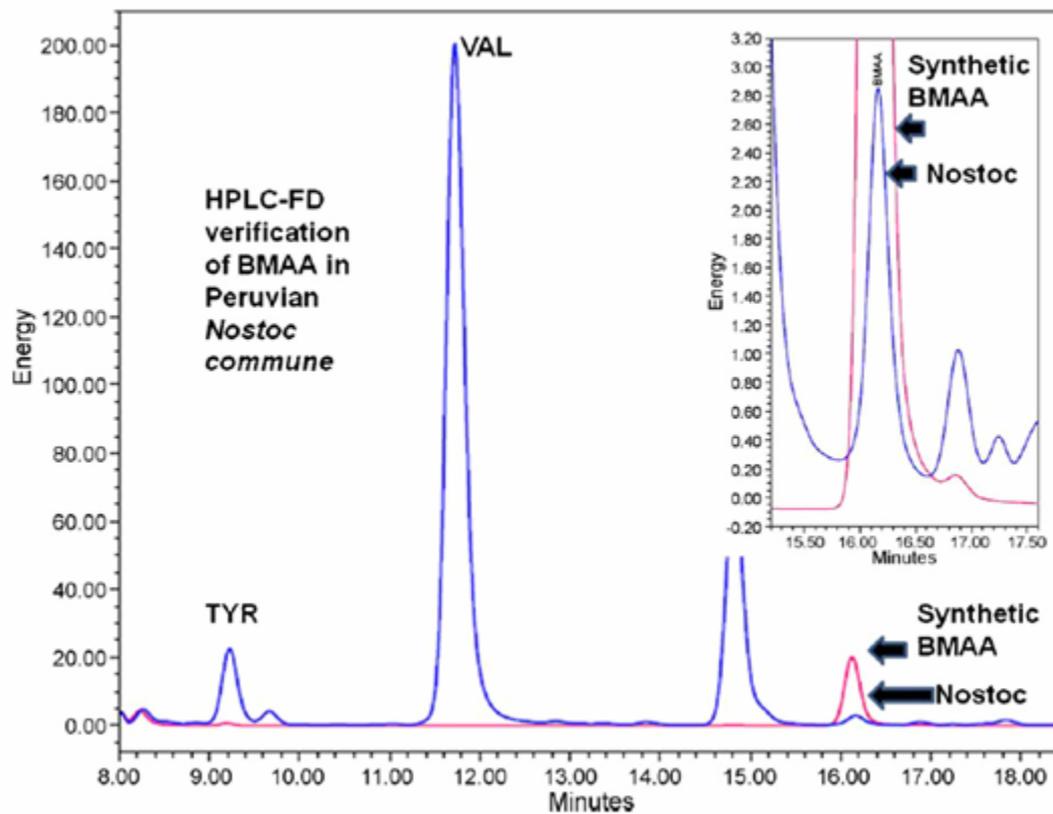
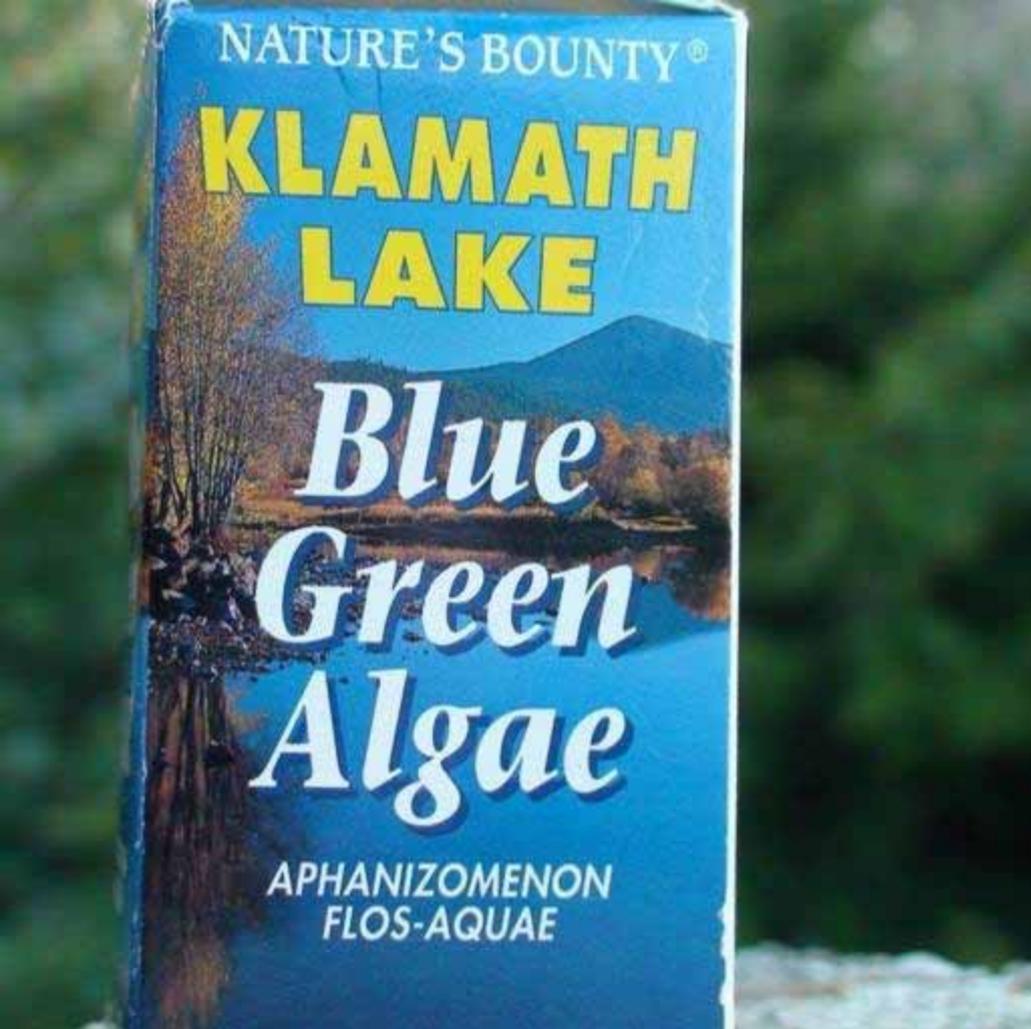


Fig. 2. HPLC chromatogram of a Peruvian Nostoc sample showing BMAA peak.

Dietary Supplements



Assessing Potential Health Risks from Microcystin Toxins in Blue–Green Algae Dietary Supplements

Duncan J. Gilroy,¹ Kenneth W. Kauffman,¹ Ronald A. Hall,¹ Xuan Huang,² and Fun S. Chu²

The presence of blue–green algae (BGA) toxins in surface waters used for drinking water sources and recreation is receiving increasing attention around the world as a public health concern. However, potential risks from exposure to these toxins in contaminated health food products that contain BGA have been largely ignored. BGA products are commonly consumed in the United States, Canada, and Europe for their putative beneficial effects, including increased energy and elevated mood. Many of these products contain *Aphanizomenon flos-aquae*, a BGA that is harvested from Upper Klamath Lake (UKL) in southern Oregon, where the growth of a toxic BGA, *Microcystis aeruginosa*, is a regular occurrence. *M. aeruginosa* produces compounds called microcystins, which are potent hepatotoxins and probable tumor promoters. Because *M. aeruginosa* coexists with *A. flos-aquae*, it can be collected inadvertently during the harvesting process, resulting in microcystin contamination of BGA products. In fall 1996, the Oregon Health Division learned that UKL was experiencing an extensive *M. aeruginosa* bloom, and an advisory was issued recommending against water contact. The advisory prompted calls from consumers of BGA products, who expressed concern about possible contamination of these products with microcystins. In response, the Oregon Health Division and the Oregon Department of Agriculture established a regulatory limit of 1 µg/g for microcystins in BGA-containing products and tested BGA products for the presence of microcystins. Microcystins were detected in 85 of 87 samples tested, with 63 samples (72%) containing concentrations > 1 µg/g. HPLC and ELISA tentatively identified microcystin LR, the most toxic microcystin variant, as the predominant congener. **Key words:** *Aphanizomenon flos-aquae*, blue–green algae, cyanobacteria, dietary supplements, microcystins, *Microcystis aeruginosa*. *Environ Health Perspect* 108:435–439 (2000). [Online 27 March 2000] <http://ehpnet1.niehs.nih.gov/docs/2000/108p435-439gilroy/abstract.html>

Klamath Lake, OR



Image State of Oregon
Image © 2009 Jackson County GIS
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Google

42°19'36.85" N 121°56'01.60" W

elev 1477 m

Jun 29, 2005

Eye alt 52.50 km



December 7, 2004

Memo: Copco Lake Analysis

To: Kier and Associates

To all concerned:

Attached below is the final Copco Lake toxin result from Wright State University. I will provide a complete summary next week, but briefly; the results show that there was a very high concentration (482 ug/L) of microcystin toxin at this shoreline station (see map on following page) on September

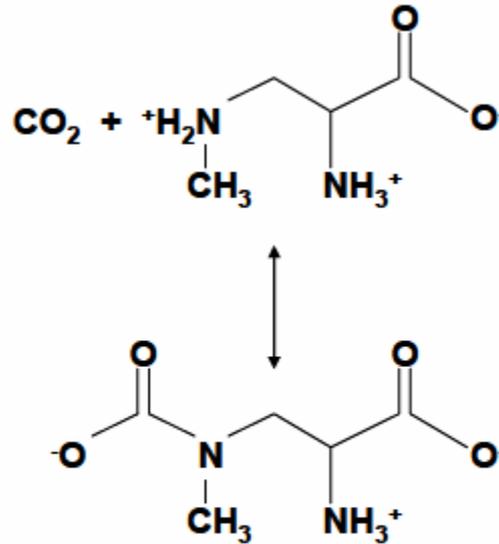
29th 2004. This is not unexpected given the 1.9 million cells/ml corresponding count of *Microcystis aeruginosa*. The microcystin level was 482 times greater than the WHO (1998) drinking water standard of 1 ug/L, but more importantly (since this is not a drinking water reservoir) this level posed a greater than moderate risk of adverse health

effects from recreational activities (Falconer et al. 1999). For example, accidental ingestion of 100 mls of lake water would have a microcystin

concentration 66 times greater than the Tolerable Daily Intake (TDI: 0.04 ug kg bw⁻¹ WHO 1998) for a 40 lb (18kg) child or 17 times greater for a 160 lb (73 kg) adult.

BMAA

β -N-methylamino-L-alanine (BMAA)



β -(N-methyl-N-carboxy)-amino-L-alanine

(from Myers and Nelson, 1990. JBC 265:10193)

BMAA and Neurological Disease

- A collaborative project:

UNH Center for Freshwater Biology

Dartmouth Hitchcock ALS Clinic

NH Department of Environmental Services

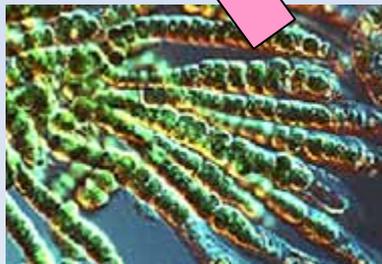


Flying Fox

Guam



Cycad tree

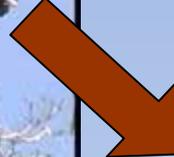


Nostoc

BMAA



Chamorros
100X neurological disease



Clusters of ALS in NH

