AQUATIC ECOSYSTEM SCIENCES LLC

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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.

Although this shoreline sample is not representative of lake-wide conditions, it does represent areas where human and pet access are likely to occur. Moreover, due to the patchy nature of blue-green algal blooms it is possible for higher *Microcystis aeruginosa* densities (and therefore higher microcystin toxin concentrations) to be present in other locations, particularly along shorelines or protected coves during calm conditions of little to no wind. Recreational users should always avoid contact with water whenever noticeable surface concentrations of algae are evident or when the lake has an obvious green to blue-green appearance. Moreover, because pets or other domestic animals are the most likely to ingest contaminated water, these animals should not be allowed access to the lakeshore whenever either noticeable surface concentrations of algae or an obvious green to blue-green appearance is evident.

Please let me know if you have any questions. Thank you.

Sincerely,

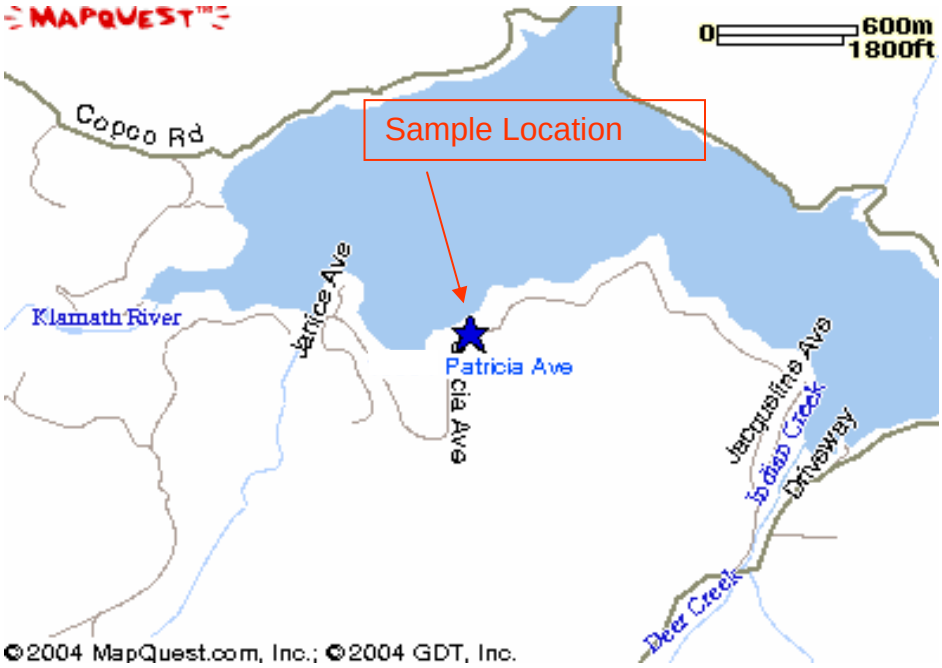
Jacob Kann, Ph.D.
Aquatic Ecologist

References

Falconer et al. 1999. Safe levels and safe practices. Pages 155-177 *in*: I. Chorus and J. Bartram, editors. *Toxic Cyanobacteria in water: a guide to their public health consequences.* World Health Organization Report. E & FN Spon, London and New York.

WHO 1998. Guidelines for Drinking-water Quality. Second Ed. Addendum to Vol. 2, Health Criteria and Other Supporting Information. World Health Organization, Geneva.

Location Map of Sample



Phytoplankton Sample Analysis

Sample: Copco Res
Sample Station: JSH
Sample Depth:
Sample Date: 29-Sep-04

Total Density (#/mL): 132,794
Total Biovolume (um^3/mL): 45,273,384
Trophic State Index: 77.3

Species	Density #/mL	Density Percent	Biovolume um^3/mL	Biovolume Percent
Nitzschia palea	120,267	90.6	21,648,000	47.8
Microcystis aeruginosa	6,514	4.9	15,269,858	33.7
Aphanizomenon flos-aquae	4,009	3.0	2,886,400	6.4
Melosira ambigua	501	0.4	4,722,471	10.4
Cryptomonas erosa	501	0.4	260,578	0.6
Nitzschia frustulum	501	0.4	60,133	0.1
Gomphonema ventricosum	501	0.4	425,944	0.9

Microcystis aeruginosa cells/mL = 1,908,732

Aquatic Analysts **Sample ID:** GW95

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November 17, 2004

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RE: Analysis Report for Sample Received 9-30-04

Sample Description and Designation: (110-1) 1 Liter Nalgene about half full, labeled "Copco Reservoir site JSH 9-29-04"

Sample Preparation.
The water sample was lyophilized and stored at -80°C prior to analysis. The sample was used for extraction and analyses of microcystins by enzyme linked immunosorbent assay (ELISA). The dried cell material was extracted twice in 20 mL of methanol. The sample was dried and fractionated by solid phase extraction (Oasis" HLB, Waters Corporation, Milford, MA). The 100 % methanol elution was used in this analysis for Microcystin determination. This fraction was dried and taken up in 1 mL of 10% (v/v) methanol. Particulates were removed by centrifugation of the sample through a Centricon YM-10 spinfilter. The filtrate was stored at -20 C and used as needed for ELISA. An Envirologix" Kit was used for analysis.

Microcystin Analysis. ELISA assay for the cyclic peptides microcystin and nodularin. The ELISA method is based upon the original polyclonal antibody method described by Chu *et al.* (1989, 1990) and adapted by An and Carmichael (1994) and Carmichael and An (1999). These methods have been adapted to a commercial ELISA kit (Microcystin Plate Kit, EP-022) that is produced by Envirologix, Inc. (Portland Maine). This Microcystin Plate Kit is calibrated to measure Microcystin concentrations between 2.5 and 0.16 μg/L. Samples containing Microcystin in greater amounts of this range are diluted. This range covers the WHO guideline level for Microcystin in finished drinking water supplies (Chorus and Bartram 1999). The minimum detectable level for Microcystin/Nodularin using this method is about 0.1 μg/L. Values below or near this level are not considered significant. Fifty microliters (50 μl) of sample, in triplicate, were used for the assay, providing a minimum detection level of 5 pg. Serial dilutions from 10^{1} to 10^{-6} (total of 32 assays) were used to run the assay.

Microcystin Results
Copco Sample

Sample	Sample Volume L	Dry Weight g	Sample Concentration μg/L MCN
110-1	**0.3 L**	**0.277g**	**482 μg/L**

Summary:

The sample showed a high microcystin content of 482 μg/L. This exceeds the WHO guideline for drinking water of 1.0 μg/L.

Further details on drinking water guideline determinations and other questions of treatment and management of toxic cyanobacteria can be found in:
Chorus, I. and Bartram, J. (eds.) Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. World Health Organization, E&FN Spon, Routledge, London, 1999.

For recreational bathing waters, there is a manual: Monitoring Bathing Waters, edited by Jamie Bartram and Gareth Rees published by E&FN Spon. for WHO and the EPA in 2000. Chapter 10 of this book is entitled "Cyanobacteria and Algae". On page 220 is a risk profile for cyanobacteria blooms in recreational waters. Moderate risk level is given as 50 μg/L chlorophyll a, 100,000 cells/L or **20 μg/L microcystin** in the top 4 meters of surface waters. Given this guideline, the water sample in this shipment presents a more than moderate risk to swimmers.

Recently, I was sent a draft copy of the Australian NHMRC Guidelines for Managing Risks in Recreational Waters (2004), which has an extensive section on Cyanobacteria. Using microcystins as a guide they have calculated risk for this toxin group in recreational waters as: Children=8 μg/L and Adults=56 μg/L. (dermal and varying amounts of accidental ingestion). This corresponds to 40,000 and 280,000 cells/ml respectively.

Signed,

Wayne W. Carmichael
Professor
Aquatic Biology/Toxicology

References:
An, J-S. and Carmichael, W.W. (1994). Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon **32**: 1495-1507.
Carmichael, W.W. and An, J-S. (1999) Using an enzyme linked immunosorbant assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. Natural Toxins. **7**: 377-385.
Chu, F.S., Huang, X., and Wei, R.O. (1990). Enzyme-linked immunosorbent assay for microcystins in blue-green algal blooms. J. Assoc. Off. Analyt. Chem. **73**: 451-456.
Chu, F.S., Huang, X., Wei, R.O., and Carmichael, W.W. (1989). Production and characterization of antibodies against microcystins. Appl. Environ. Microbiol. **55**: 1928-1933.

Samples are run on a Molecular Devices Corp., Vmax kinetic microplate reader, Palo Alto, CA.