2. Project Description

A) Statement of Water Quality Concerns/Issues

Freshwater cyanobacteria blooms pose a serious threat to human and ecological health as well as creating negative economic impacts. The occurrence of fresh water cyanobacteria blooms is a worldwide phenomenon that is believed to be amplified by global climate change and anthropogenic impacts. One third of the fifty genera of freshwater cyanobacteria are capable of producing toxins. Recognizing the potential health risk, Canada, the United Kingdom, Australia, and many European countries have established guidelines for the level of cyanobacterial toxins permissible in drinking and recreational waters. The United States has acknowledged cyanotoxins as a potential health risk by placing them on United States Environmental Protection Agency (US EPA) drinking water Contaminant Candidate List (CCL I, CCL II and proposed CCL III). In 1999 a cyanotoxin priority list was created for the CCL; the list includes microcystin congeners LR, RR, YR, and LA, cylindrospermopsin, and anatoxin-a. Recent research preformed by NOAA, USEPA, USGS, USDA, and academic institutions show nation wide impairment of freshwater sources[1].

In order to determine the health risk of a drinking or recreational water contaminant, ample reliable data is needed in the areas of occurrence, toxicity and fate. The monitoring of inland Michigan lakes for cyanobacteria and their toxins has been minimal[2, 3], although the cyanobacteria profiles and high microcystin levels of Bear, Mona and Muskegon Lakes has brought Michigan national recognition which included a human exposure study by Center of Disease Control [1]. Currently, much of the cyanotoxin monitoring is being performed on Lakes Erie, Michigan, and Huron, on a small group of western Michigan inland lakes, and on Ford Lake in Washtenaw County. Microcystin is reported as the most common algal toxin in United States[1] and to large extent this is because there are several genera of cyanobacteria that produce microcystin and microcystin producing cyanobacteria are prevalent in all climates. In the last ten years, the topical/subtoprical cyanobacteria species *Cylindrospermopsis raciborskii* has been reported in several of the northern Midwestern states; Indiana, Wisconsin, Ohio, and Michigan. The algal profile of a summer 2007 Kent Lake sample reported both coiled and straight *Cylindrospermopsis raciborskii*. Prior to this report only four lakes in Michigan have been documented with *Cylindrospermopsis*; two lakes in the MDEQ 2005 Western Inland Lake Study, Lake Erie and Lake Huron. As part of the 2004 and 2007 Kent Lake aquatic ecology study, Dr. Pullman had qualitative algae analyses performed. In comparing the 2004 to 2007 list of algae, the noted difference was the appearance of *Cylindrospermopsis raciborskii*. The potential risk is that some strains of *Cylindrospermopsis raciborskii* are known to produce cylindrospermopsin, a cytotoxin. Cylindrospermopsin exposure through ingestion can lead to liver and kidney damage. More recently, studies have suggested that cylindrospermopsin is genotoxic and carcinogenic. A USEPA NCEA draft toxicological reviews suggests a subchronic reference dose of 0.03 ug/kg/bw/day for cylindrospermopsin and anatoxin-a, which leads to a drinking water MCL of about 1.0 ug/L. From an ecological perspective this cyanobacterium appears to reduce the size and diversity of zooplankton and phytoplankton[4-6].

Several recreational lakes in Washtenaw County have favorable cyanobacteria growth conditions; high temperature, low flow, high phosphorus levels and high phosphorus to nitrogen ratio. This proposal is a joint monitoring project between Clinton Huron Metroparks and Washtenaw County Health Department to monitor Kent Lake, Portage and Ford Lakes downstream from Kent Lake, Silver, Sugarloaf, Half Moon, Bruins and Independence Lakes to determine the *Cylindrospermopsis* and cyanobacteria and their toxin profile during late summer. The proposal includes cyanobacteria identification/enumeration and quantification of the priority toxins and not just *Cylindrospermopsis* and cylindrospermopsin because there is only a minimal cost increase (5% for chemicals) for the complete analyses. Data presented from western inland MDEQ and Ford Lake studies suggest that late July and August will be the best monitoring months.

The results from this study will be used by Washtenaw County Health Department and the Huron Clinton Metroparks to assist in evaluating the potential risk of nontoxic and toxic *Cylindrospermopsis* and other cyanobacteria blooms. This proposed project will provide both organizations with tools and training to identify high risk locations such as shallow downwind bays, skills to grab valid samples, and guidance on interpreting future data. As part of this project, a cyanotoxin recreational water contingency plan will be developed, so that if either of these two agencies finds themselves with a toxic cyanobacteria bloom, proper precautions are in place to minimize human and domestic animal exposure.

B) Project Goals and Objectives

The primary goal of this project is to quantify *Cylindrospermopsis raciborskii* and its toxin, cylindrospermopsin and create a profile for several recreational lakes in Washtenaw County and Kent Lake. A secondary goal is to determine the cyanobacteria and cyanotoxin profile of the proposed lakes.

Objective I: To review the proposed eight lakes nutrient data, local prevailing winds, land use, recreational use, and historical water quality data. Most human and domestic animal exposures occur when cyanobacteria blooms are concentrated on the shores of beaches and other recreational areas. The literature review will include searching through federal and state documents, such as Army Corp. of Engineers. A short review will be written on each lake. Based on the review information, sampling locations will be determined.

Objective II: Sampling the selected sites on each lake. Sites will be identified by coordinates using decimal degrees and Michigan Center for Geographic Information basemap layers. Lake will be sampled from mid July to mid August. The final report will include maps with sites and its coordinates.

Objective III: Determine the occurrence and abundance Cylindrospermopsis and its toxin, cylindrospermopsin, in the proposed lakes.

Objective IV: Determine the occurrence and abundance of cyanobacteria genera and the priority cyanotoxins.

Objective V: After the first sampling which will take place late July to mid August, data will be evaluated according to the WHO monitoring strategy for freshwater cyanobacteria to determine if sampling should continue as planned or if the resources should be reallocated to monitor moderate and high risk locations.

Objective VI: To draft a recreational water cyanotoxin contingency plan for Washtenaw County and the Huron Clinton Metroparks. The draft contingency plan will include action levels for each of the cyantoxins, a plan to disseminate the no recreation/swimming notice, a posting and fact sheet for the websites, a decision tree for monitoring, and a list of analytical assays and analyses with contact information.

Since *Cylindrospermopsis* can have both environmental and human health impacts, the extent and presence *Cylindrospermopsis* in Michigan inland lakes needs to be known. Each lake will be assigned a risk level for *Cylindrospermopsis* toxic cyanobacteria blooms. This information can be used by the agencies for future monitoring and lake mitigation. The proposed study will allow these agencies and MDEQ to better understand inland lake cyanobacteria profiles and to provide a model for the development of a recreational waters cyanotoxin contingency plan.

C) N/A

D) Monitoring Sites

Kent Lake, Livingston County (42.5289,-83.6466)

Kent Lake is surrounded Kensingston Park, Huron Clinton Metropark. Kent Lake has reported the following cyanobacteria genera: *Microcystis*, *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Planktolyngbya*, *Pseudanabaena*, and *Cylindrospermopsis*. Several of these genera are capable of producing toxins.

Portage Lake, Washtenaw County (42.4233, -83.9161)
No cyanobacteria/cyanotoxin data found in the literature.

Ford Lake, Washtenaw County (42.2177, -83.5902)

Ford Lake was part of an USEPA STAR and CSREES study performed by Dr. Lehman. This study reported the presences of both Microcystis and the microcystin (ref).

Silver Lake, Washtenaw County (42.4191,-83.9607)
No cyanobacteria/cyanotoxin data found in the literature.

Independence Lake, Washtenaw County (42.4063, -83.8025)
No cyanobacteria/cyanotoxin data found in the literature.

Sugarloaf Lake, Washtenaw County (42.3420,-84.1148)
No cyanobacteria/cyanotoxin data found in the literature.

Halfmoon Lake, Washtenaw County (42.420223,-84.012426)
No cyanobacteria/cyanotoxin data found in the literature.

Bruin Lake, Washtenaw County (42.419109,-84.038674)
No cyanobacteria/cyanotoxin data found in the literature.

E) Organization Information

Washtenaw County currently has four lakes with special assessment districts, through P.A. 185 to monitor and manage the water quality for maintain biodiversity and maintain recreational capabilities. Monitoring water quality through routine sampling of bathing occurs every summer. Website videos have been produced to educate citizens on their affect on water quality has also been developed (go to www.ewashtenaw.org/ep and click on the green media button). The County has retained Dr. Pullman to assist in management issues for inland lakes, he has provided management opinions for lake communities with water quality concerns.

F) Partners

Washtenaw County will provide staff to collect the samples. Dr. Pullman will provide training in sampling procedures and assist in collecting samples. Washtenaw County will also post results of information of sample results on its lakes management webpage.

Dr. Judy Westrick, an assistant professor at Lake Superior State University and Direct of the Lake Superior State University Environmental Analytical Laboratory, has designed, coordinated and performed several cyanobacteria and cyanotoxin monitoring projects; Great Lakes source waters (2008, US EPA), three national source and drinking waters studies (2002, 2004, and 2005 US EPA), St. John River Management Project (2005-2010), Ohio River (1995-2001, ORSANCO) and several private community monitoring programs. Dr. Westrick has organized, moderated, and presented at national and international harmful algal bloom conferences. Dr. Westrick understands that with increased numbers of cyanobacteria blooms come environmental and human health concerns as well as economic consequences.   Dr. Westrick directs of LSSU Environmental Analytical Laboratory.

The Lake Superior State University Environmental Analysis Laboratory (LSSU EAL) is located in Michigan's Eastern Upper Peninsula and is part of the Division of Chemistry, Environmental Science, Geology, and Physics. The LSSU EAL is equipped with modern instrumentation allowing us to perform a variety of chemical analyzes in a timely manner adhering to stringent quality control / quality assurance protocols. The LSSU EAL has worked on numerous cyanobacterial studies on both a local and national level coordinating and performing sampling, analysis, reporting, and the logistics of the entire process. We serve local, state, and federal agencies to ensure both regional and national research and monitoring needs are met.

G) Project Sustainability

Washtenaw County will administer lake management projects as developed through P.A. 185. We will continue to collect all pertinent information regarding ecological and biological health of lakes on the County's lake management website. The county will collect and provide information generated by other organizations such as the MDNR, Huron River Watershed Council, the Office of the Water Resource Commissioner of Washtenaw County and other agencies with relevant information to help citizens maintain water quality.

H) Evaluation

Each lake will be classified at a low, moderate or high risk cyanobacteria bloom lake. The lakes will be classified according to the WHO Monitoring Guidelines presented in "Monitoring Bathing Waters". The historical data, cyanobacteria identification and enumeration, and toxin levels will be used to determine the risk factor. The WHO Monitoring Guidelines are divided into 3 phases Phase 1: background, Phase 2: basic, and Phase 3: cyanobacteria.   Phases 1 is to determine the rationale for monitoring and Phase 2 is a site inspection for the indicators. Both of these will be accomplished by reviewing the historical and physical data. Phase 3 is a qualitative/quantitative assessment of potentially toxic cyanobacteria assemblages.   Phase 3 is divided into low, moderate and high risk based on the cells of cyanobacteria per mL.

3) Work Plan

Task 1: Dr. Westrick and a student will collect the historical and physical data for the eight lakes. The relevant data will be placed into a table and an initial risk factor will be assigned to each lake. From these data sampling locations will be determined.

Task 2: Collect samples mid July to mid August. Two one liter Samples will be collected from the euphotic zone from each location. Dr. Doug Pullman will coordinate and supervise the sample collections. Two liters of sample will be collected in glass bottles. Samples will remain on ice and shipped by next day air to the LSSU Environmental Laboratory.

Task 4: Samples will be logged and aliquots for cyanotoxin and identification/ enumeration analyses.

Task 5: Cyanobacteria identification/enumeration.
Water samples will be settled in chambers for at least 24 hours (Wetzel and Likens, 1991). Lugol's iodine will be added to maintain preservation and assist with identification and settling. A qualitative and quantitative analysis will be performed at 200X using a Wild M40 inverted microscope. Algal identifications will be made using standard taxonomic references such as Prescott (1982)[7]. A minimum of 300 cyanobacteria cells will be counted per sample. This method is a slightly modified version of the methods published by the World Health Organization (WHO) [8]. The major difference is that the WHO suggests counting between 400 and 800 specimens giving the maximum error for the total count between 7-10 percent whereas we have chosen to count at least 300 algal cells or 300 fields giving the maximum error for the total count between 10-15 percent. This modification lowers the analysis cost by approximately 40% and does not significantly impact policy and action levels.

Task5: Intracelluar priority cyanotoxins levels by HPLC/PDA.

Glass Fiber Filter Extraction
The glass fiber filter sample preparation procedure can be use to extract and concentrate intracellular algal toxins. This method will extract hydrophilic toxins like anatoxin-a and cyclindrospermopsin and hydrophobic toxins like microcystin LR and LW by using two 50:50 methanol/water and two 85:15 methanol/water extractions[9](Brenton 2001, Hiripi 1998). Filtered cells are initially lysed by three freeze/thaw cycles and sonicated between each extraction.

HPLC Analysis for Microcystins and Cylindrospermopsin

The high pressure liquid chromatography (HPLC) method currently used is specific for the microcystin LR, RR, LA and YR (based on availability of standards), anatoxin-a and cylindrospermopsin. This method uses an acetonitrile and water gradient containing 0.02% trifluoroacetic acid utilizing an ultraviolet detector (UV). Nodularin will be used as the internal standard since it is produced by brackish water cyanobacteria (Nodularia) [10, 11] (Akin-Oriola, 2005).

Washtenaw County along with Dr. Pullman will produce and submit quarterly reports and final report.

Products and deliverables

Qualitative cyanobacteria identification and quantitative intracellular cyanotoxin analyses will be performed within 72 hours of the time sample was received and results will be reported to the agencies within the next 48 hours. Quantitative cyanobacteria identification/enumeration will be performed within 3 weeks and reported within the next 48 hours.

QAPPs will be made available within three weeks after the grant is funded.

Timeline

Month	Timeline	Percentage of Time
April 30, 2009	Schedule meeting with Jeff K, Doug Pullman, a representative from HCMP and Drs. Judy Westrick and Dave Szlag	5%
May 30, 2009	Write short review on each lake.	10%
June 30, 2009	Quarterly Report, Review of Historical Data and Report Monitoring Sites	5%
July 30, 2009	Mid July to Mid August initial monitoring is completed. Data are evaluated and either less sites are monitored more frequently or the sample collection is repeated.	50%
September 12, 2009	Sample is complete	
November 30, 2009	Quarterly Report and Draft of Final Report	20%
December 30, 2009	Final Report to MDEQ	10%

1. Hudnell, H. K., Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. *Advances in experimental medicine and biology* **2008,** 619, n.
2. Hong, Y. S. A.; Biddanda, B. R. R.; Fahnenstiel, G., NOTE - Occurrence of the toxin-producing cyanobacterium Cylindrospermopsis raciborskii in Mona and Muskegon lakes, Michigan. *Journal of Great Lakes research* **2006,** 32, (3), 8.
3. Steinman, A.; Rediske, R.; Denning, R.; Nemeth, L.; Chu, X.; Uzarski, D.; Biddanda, B.; Luttenton, M., An environmental assessment of an impacted, urbanized watershed: the Mona Lake Watershed, Michigan. *Archiv fèur Hydrobiologie* **2006,** 166, (1), 117.
4. Leonard, J.; Paerl, H., Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St Johns river system, Florida as influenced by the toxic cyanobacterium Cylindrospermopsisraciborskii. *Hydrobiologia* **2005,** 537, (1-3), 89-97.

5. Dobberfuhl, D. R., Cylindrospermopsis raciborskii in three central Florida lakes: population dynamics, controls and management implications. *Lake and Reservoir Management* **2003,** 19, (4), 341-348.
6. Borics, G.; Grigorszky, I.; Szabâo, S.; Padisâak, J., Phytoplankton associations in a small hypertrophic fishpond in East Hungary during a change from bottom-up to top-down control. *Hydrobiologia* **2000,** 424, (1), 12.
7. Prescott, G. W., *Algae of the Western Great Lakes Area*. Ottokoeltz Science Publishers: Loenigstein, West Germany, 1982.
8. Bartram, J. F.; Chorus, I., *Toxic Cyanobacteria*. EF&N Spon: 1999.
9. Barco, M.; Lawton, L. A.; Rivera, J.; Caixach, J., Optimization of intracellular microcystin extraction for their subsequent analysis by high-performance liquid chromatography. *Journal of Chromatography* **2005,** 1074, 23-30.
10. Oehrle, S. A.; Westrick, J., Analysis of Various Cyanobacterial Toxins by LC-MS. *LCGC North America* **2003,** 21, (7).
11. Bogialli, S.; Bruno, M.; Curini, R.; Di Corcia, A.; Lagana, A.; Mari, B., Simple Assay for Analyzing Five Microcystins and Nodularin in Fish Muscle Tissue: Hot Water Extraction Followed by Liquid Chromatography-Tandem Mass Spectrometry. *Journal of agricultural and food chemistry* **2005,** 53, (17), 7.

Michigan Department of Environmental Quality
Water Bureau

GRANT APPLICATION BUDGET
(Authorized by 1994 PA 451)
(Completion of this form is required in order to receive grant consideration)

Applicant Name: Washtenaw County Environmental Health
Project Name: Determining the prevalence of Cylindrospermopsis in 7 Washtenaw County Lakes & Kent Lake
Project Dates: April 2009 to December 31, 2009

Column 1	Column 2	Column 3	Column 4 **GRANT AMOUNT**	Column 5 **LOCAL MATCH AMOUNT**	Column 6 **TOTAL**
STAFFING					
NAME	**HOURS**	**RATE**			
Judy Westrick	160	$ 35.00	$ 5,600.00	$ -	$ 5,600.00
Jeff Krcmarik, Washtenaw County Environmental Health Sup	80	$ 42.72	$ -	$ 3,417.60	$ 3,417.60
Julie Rowe , Environmental Technician	40	$ 22.24	$ -	$ 889.60	$ 889.60
Dr. Doug Pullman, Aquest Inc	25	$ 100.00	$ -	$ 2,500.00	$ 2,500.00
enter name and title	0	$ -	$ -	$ -	$ -
enter name and title	0	$ -	$ -	$ -	$ -
STAFFING Subtotal			$ 5,600.00	$ 6,807.20	$ 12,407.20
FRINGE BENEFITS (not to exceed 40%)					
NAME	Salary	**RATE**			
Judy Westrick	$ 5,600.00	20%	$ 1,100.40	$ -	$ 1,100.40
Jeff Krcmarik	$ 3,417.60	40%	$ -	$ 1,367.04	$ 1,367.04
Julie Rowe , Environmental Technician	$ 889.60	40%	$ -	$ 355.84	$ 355.84
Dr. Doug Pullman, Aquest Inc	$ 2,500.00	0%	$ -	$ -	$ -
enter name and title	$ -	0%	$ -	$ -	$ -
enter name and title	$ -	0%	$ -	$ -	$ -
FRINGE BENEFITS Subtotal			$ 1,100.40	$ 1,722.88	$ 2,823.28
STAFFING & FRINGE BENEFITS Subtotal			**$ 6,700.40**	**$ 8,530.08**	**$ 15,230.48**
INDIRECT RATE (not to exceed 20% Staffing and Fringe Benefits)					
INDIRECT COSTS		**RATE**			
List items included in indirect costs here!		20%	$ 1,340.08	$ 1,706.02	$ 3,046.10
CONTRACTUAL SERVICES					
NAME	**# samples**	**per sample**			
LSSU	80	$ 200.00	$ 16,000.00		$ 16,000.00
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
CONTRACTUAL SERVICES Subtotal			$ 16,000.00	$ -	$ 16,000.00
SUPPLIES, MATERIALS, & EQUIPMENT					
SUPPLIES & MATERIALS (itemize)	**QUANTITY**	**COST**			
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
SUPPLIES & MATERIALS Subtotal			$ -	$ -	$ -
EQUIPMENT	**QUANTITY**	**COST**			
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
		$	$ -	$ -	$ -
EQUIPMENT Subtotal			$ -	$ -	$ -
SUPPLIES, MATERIALS, & EQUIPMENT Subtotal			$ -	$ -	$ -
TRAVEL	**MILES**	**RATE**			
MILEAGE			$ -	$ -	$ -
	NIGHTS	**RATE**			
LODGING	4	$ 125.00	$ 420.00	$ -	$ 420.00
	0	$ -	$ -	$ -	$ -
MEALS	18	$ 10.00	$ 180.00	$ -	$ 180.00
	0	$ -	$ -	$ -	$ -
OTHER (itemize) mileage	1200	$ 0.51	$ 600.00	$ -	$ 600.00
TRAVEL Subtotal			$ 1,200.00	$ -	$ 1,200.00
GRAND TOTALS			$24,140.08	$ 10,236.10	$ 34,376.18

Local Match must be at least 25% of project total which is $ 8,594.04

Michigan Department of Environmental Quality
Water Bureau

GRANT APPLICATION BUDGET
(Authorized by 1994 PA 451)
(Completion of this form is required in order to receive grant consideration)

Applicant Name: Washtenaw County Environmental Health
Project Name: Determining the prevalence of Cylindrospermopsis in 7 Washtenaw County Lakes & Kent Lake
Project Dates: April 2009 to December 31, 2009

Column 1	Column 2	Column 3	Column 4 **GRANT AMOUNT**	Column 5 **LOCAL MATCH AMOUNT**	Column 6 **TOTAL**
SOURCES OF MATCH:	**DOLLAR VALUE COMMITTED:**				
				$ -	
				$ -	
				$ -	
				$ -	
				$ -	
				$ -	
TOTAL MATCH				$ -	

(Revised 11-04)