SAMPLING AND ANALYSIS PLAN FOR TOXIC CYAONOBACTERIA IN LAKE WASHINGTON, LAKE SAMMAMISH, AND LAKE UNION

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1 Introduction

King County plans to collect samples for analysis of the cyanotoxin, microcystin, in selected King County lakes during summer and fall of 2002 through 2004. This Sampling and Analysis Plan (SAP) describes the planned scope of work, field sampling procedures, and laboratory analytical requirements for the project.

Mass accumulations or "blooms" of cyanobacteria in freshwater ecosystems are primarily caused by nutrient, particularly phosphorus (P), enrichment. Cyanobacteria blooms can cause surface scums, decreased water column transparency, dissolved oxygen depletion, and unpalatable drinking water due to taste and odors. Some cyanobacteria also produce toxic compounds ("cyanotoxins") that have caused livestock, wildlife, and pet fatalities worldwide (reviewed by Carmichael 1994; Chorus 2001).

Cyanotoxins include a broad, diverse range of chemicals and mechanisms of toxicity (Carmichael 1994; Sivonen and Jones 1999). Major classes of cyanotoxins include the cyclic peptides, which are primarily hepatotoxins (microcystins and nodularins); alkaloids and an organophosphate, which are strong neurotoxins (anatoxin-a, anatoxin-a(S), and saxitoxins); a cyclic guanide alkaloid, which inhibits protein synthesis (cylindrospermopsin); lipopolysaccharides, which have pyrogenic properties; and dermatoxic alkaloids (aplysiatoxins and lyngbyatoxins) (Chorus 2001).

The hepatotoxins are of particular concern due to their prevalence and potential to harm animals and humans. Hepatotoxins damage liver tissues, and at high doses can cause liver failure and death (Carmichael 1994). Hepatotoxins with seven amino acids are called microcystins (produced by species of *Microcystis, Planktothrix, and Anabaena*) and those with five amino acids are nodularins (produced by *Nodularin spumigena*). The mechanism of toxicity involves the inhibition of the specific protein phosphatase enzymes possessed by all eukaryotic cells. In addition, microcystins are suspected tumor-promoters and teratogens (Falconer 1998). These toxins have been associated with elevated rates of primary liver cancer in people drinking waters with high densities of cyanobacteria (Yu 1989).

In a comparison of surveys from different countries, Chorus (2001) found that microcystins were more frequently detected than anatoxins in cyanobacterial bloom samples. Microcystins were detected in at least 60% of all samples investigated in the Danish, German, Portuguese and Korean studies. In the Czech study, microcystins were detected in 90% of all samples. Neurotoxicity was documented in one quarter or less of the bloom samples investigated in these studies (Chorus 2001). Thus, investigations of cyanotoxicity and the development of public health guidelines have focused on microcystins due to their widespread occurrence and potential for chronic toxicity.

1.1 Project Background

Toxic cyanobacteria have been increasingly detected in western Washington lakes since the first documented toxic episode in American Lake in 1989 (Jacoby et al. 1994). Since then, toxic cyanobacteria have been detected in Steilacoom Lake (Jacoby et al. 2000),

American Lake, Green Lake, Lake Waughop, and Lake Sammamish resulting in several lake closures. Microcystins were measured at concentrations of approximately 500 µg g-1 dry weight cyanobacteria and may have been responsible for the death of a pet dog and the reported illnesses of several young children who swam in Lake Sammamish during a toxic bloom in September 1997.

The toxic bloom of *Microcystis aeruginosa* in Lake Sammamish during 1997 and subsequent detection of microcystins by Johnston and Jacoby (in press) in 1999 prompted King County to develop a sampling and analysis plan for the measurement of microcystins in other King County lakes.

1.2 Summary of Previous Studies

Few studies of cyanotoxicity have been conducted in lakes in Washington State (e.g., Jacoby *et al*. 1994; Jacoby *et al*. 2000). Cyanobacterial activity and environmental conditions that may promote toxic cyanobacteria were investigated in Lake Sammamish during summer and fall 1999 (Johnston and Jacoby in press). Microcystins were detected using ELISA during late August and early September 1999 despite low cyanobacterial abundance. Microcystin concentrations ranged between 0.19–3.8 µg L^{-1} at all depths throughout the lake with the exception of the boat launch where a surface concentration reached 43 µg L^{-1}.

During the toxic episodes in 1997 and 1999, *Microcystis* was associated with a stable water column, increased surface total phosphorus concentrations (> 10 µg L^{-1}), surface temperatures greater than 22°C, high total nitrogen to phosphorus ratios (> 30), and increased water column transparency (up to ~5.5 m). Migration of *Microcystis* and *Anabaena* occurred in both the deep and shallow portions of the lake. Migration rates were more than 2 times higher at the shallow station, and the migrating cyanobacteria were dominated *by Microcystis* (89-99% of the total biovolume) at both stations. External loading of nutrients due to the large storm event that preceded the 1997 toxic episode may have provided the nutrients needed to fuel that bloom. Despite the lack of rain and subsequent external runoff, toxic *Microcystis* occurred in 1999. The migration of *Microcystis* from the nutrient-rich sediments may have contributed to the toxic population detected in 1999.

1.3 Regulatory Status of Cyanotoxin Criteria and Guidelines

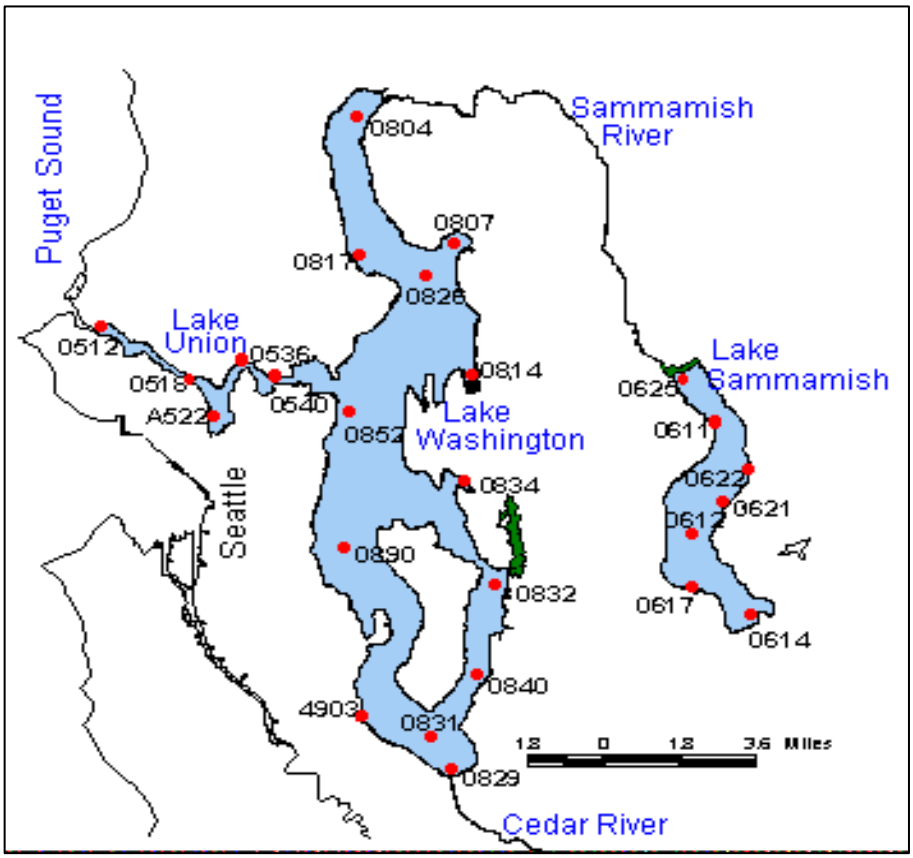
The U.S. Environmental Protection Agency, the State of Washington Department of Ecology (Ecology), and the Washington State Public Health Department have not promulgated water quality standards for cyanotoxins. Thus, the increased detection of cyanotoxins in water bodies worldwide generates a complex challenge for water resource managers. The World Health Organization (WHO) has proposed that microcystin concentrations should not exceed 1 µg L^{-1} in drinking water (WHO 1998; Chorus & Bartram 1999).

Chorus (2001) performed a risk assessment on how much scum could kill a small child based on a worst-case scenario using toxicological data and microcystin concentrations. In this assessment, she assumed that the acute oral toxicity of microcystin-LR in mice (LD_{50} 5-10.9 mg kg^{-1} body weight) applies to humans and an intracellular microcystin concentration of 3 mg mL^{-1} of cell material was present in the scum. For a 10 kg child, 50 mg microcystin could be a lethal dose, which would correspond to 17 mL of pure cell material. Extending this analysis to a high microcystin concentration of 24 mg L^{-1} (measured in a scum sample from Germany), Chorus (2001) estimated that a toxic dose for a 10-kg child would require swallowing about 2 L of water. If the scum is more toxic or the cell density of *Microcystis* in the scum is higher, a lethal dose could occur at lower volumes of ingested water (e.g., with a fivefold increase in toxicity and a four-fold higher cell density, 0.1 L of water could be lethal). She concludes that although acute microcystin poisonings are possible at the concentrations measured in recreational water bodies, they are unlikely. However, liver damage in people exposed to high microcystin concentrations during swimming is not unlikely, particularly if exposure is repeated or prolonged. The implications are most severe for children who tend to play in shallow areas where dense scums accumulate. The long-term consequences of these exposures in terms of liver damage and human health are uncertain.

Several countries have developed guidelines for recreational exposure to cyanotoxins. The focus of these guidelines is on microcystins due to their widespread occurrence and potential for chronic toxicity (Chorus *et al*. 2000; Chorus 2001). For example, the Federal Environmental Agency of Germany recommends posting warning signs and conducting a remedial investigation if chl-*a* exceeds 40 µg L^{-1} and cyanobacteria are dominant. If chl-*a* exceeds 150 µg L^{-1} or if total microcystin concentrations exceed 100 µg L^{-1}, closure of the swimming beach is recommended until the bloom declines. WHO has issued guidelines that identify three levels of hazards based on cyanobacteria abundance as measured by concentrations of chl-*a* or cell densities (Fastner *et al*. 1999). However, the large variability in the microcystin:chl-*a* ratios found in several studies (reviewed by Chorus 2001) indicates that direct measurement of microcystins is a more accurate measure of the potential toxicity of cyanobacteria. In this regard, microcystin:chl-*a* ratios in Lake Sammamish ranged from 0.4 to 6.4, with higher ratios (mean = 3.20) in the hypolimnion during the toxic episode in 1999 (Johnston and Jacoby in press).

1.4 Study Area Description

Initial routine sampling for microcystin analysis using the ELISA procedure was initiated in spring of 2002. This SAP describes a more focused and thorough approach that will be implemented for selected sites beginning in May, 2003, and will be conducted along with the ongoing routine sampling. This focused study will be conducted in lakes Washington, Sammamish, and Union at selected locations currently active in the Major Lakes monitoring program (see Major Lakes Monitoring Program SAP, King County, forthcoming in 2003). If blooms are detected in other regional lakes, additional grab samples may be collected from these lakes and analyzed. If bloom samples are collected at other than established stations, coordinates will be collected.



Sampling Sites for the Major Lakes Monitoring Program

1.5 Project Objectives

The objectives of the Toxic Cyanobacteria in King County Lakes Study are to:

- Conduct a baseline screening study of microcystin concentrations in selected King County lakes during spring through fall
- Establish relationships between microcystins and other indicators of cyanobacteria biomass [e.g., chlorophyll *a* (chl-*a*), cell densities, fluorometer readings]
- Assess human health risks associated with exposure to microcystins during recreational use of King County lakes.

The study will provide decision-makers with information and recommendations regarding recreational water use during cyanobacterial blooms and will lead to improved management of King County lakes for the protection of aquatic life and human health.

2 Study Design

2.1 Approach

This survey is primarily designed to evaluate the potential for cyanobacterial toxicity and the presence/absence of cyanobacterial toxins, and secondarily to estimate concentrations and geographic extent of the toxicity, should it be present. The assessment of cyanotoxins will focus on microcystins due to their widespread occurrence and potential for chronic toxicity. Microcystins will be measured in three water bodies in King County's Major Lakes Program (i.e., Lakes Sammamish, Washington and Union). Measurement of microcystins will coincide with the routine water quality monitoring in these lakes beginning in March 2003. After two years of monitoring microcystins, the monitoring program will be re-evaluated and the sampling design optimized.

Three (3) types of sample collection scenarios are included in this study.

1. Routine Major Lakes Sampling. Sampling sites will be selected at routine lake monitoring locators in order to relate cyanobacterial data to other lake data. These routine stations include 0831, 0826, and 0852 in Lake Washington, 0611 and 0612 in Lake Sammamish, and A522 in Lake Union. Samples are typically collected once per month between October and February, and twice per month between March and September. The routine sampling program is summarized in Table 1.

   An aliquot of the composite sample collected as part of the routine sampling effort will be used for this analysis. This composite will be an integrated sample from the surface to a 10-meter depth. The approach for collecting integrated composite samples is described in the Major Lakes Quantitative Phytoplankton Study SAP, King County, 2003. In order to obtain sufficient volume for the Toxic Cyanobacteria Study tests, the sampling apparatus may need to be modified to allow simultaneous submersion of two or more tubes, or, multiple tube deployments may be needed. In the case of multiple tube deployments, individual aliquots will be composited onboard in a clean, stainless steel bucket prior to filling sample containers.

   Phytoplankton identification and qualitative enumeration, microcystin analysis by ELISA and chl-*a*/pheo-*a* (pheophytin *a*) analysis is conducted on all samples as part of the Major Lakes Routine Monitoring program. (See Major Lakes Monitoring Program SAP for further discussion). Note that quantitative phytoplankton enumeration and identification is also being performed for samples collected from these 6 stations, as part of the SWAMP Major Lakes Quantitative Phytoplankton Study, and the additional, more focused Toxic Cyanobacteria Study analyses will be performed for the 6 selected stations as well. Pheo-*a*, a photo-degradation product of chl-*a*, will be measured to allow differentiation of these pigments from the chl-*a*. The chl-*a* concentrations as reported by the laboratory will have already been corrected for any pheo-*a* that may be present.

2. Bloom Sampling. Focused sampling efforts will be made to collect scums or accumulations of cyanobacteria if they are present within the visual distance of routine sampling sites (see 1 above). A bloom will be defined by a visually observable accumulation of phytoplankton in the water column or as a surface accumulation. These grab samples will be given their own coordinates and a LIMS locator created. New locator names will be consistent with the naming convention system established for the Major Lakes. Up to 10 samples may be collected during a bloom event, at which time the Toxic Cyanobacteria Study Project Manager will evaluate such data as is available and discuss with the laboratory available options for proceeding with the bloom investigation.

   Sufficient volume will be collected for toxicity testing, chl *a/*pheo-*a*, and phytoplankton quantitative enumeration and identification. Mycrocystins will be measured by ELISA and PPIA on these discrete samples. If toxins are present, chl–*a*/pheo-*a* and quantitative phytoplankton identification and enumeration will be determined using the same methodology as for the Routine Major Lakes sampling effort. (See Major Lakes Monitoring Program SAP for further discussion).

3. Swimming Beach Monitoring. The third sampling scenario will be conducted by the laboratory's Environmental Services Section (ESS) staff as part of the swimming beaches monitoring program. ESS will routinely inspect the waters visually for cyanobacteria blooms when conducting the routine beach monitoring program. Visual aids, e.g., photographs, field guides, will be provided to ESS by the Project or Program Manager to assist with this inspection.

   As with Bloom Sampling (see 2 above), up to 10 samples may be collected per bloom event, followed by Project Manager evaluation, and subsequent decisions regarding appropriate next steps. Sufficient sample volume will be collected for microcystin testing, chl-*a/*pheo-*a* analysis, and quantitative phytoplankton identification and enumeration if necessary. Sample collection will be conducued as a surface dip.

Table 1. Major Lakes Routine Sampling Design Summary – Toxic Cyanobacteria

Lake Sammamish			
Routine Composite Stations	Number of Samples per Visit	Number of Visits per Year	Total Number of Samples per Year[a]
0611	1	19	19
0612	1	19	19
Lake Washington			
0826	1	19	19
0852	1	19	19
0831	1	19	19
Lake Union			
A522	1	19	19
Field Replicate	One station (0852) every other event	9	9
Laboratory Replicate	One station (0852) every other event	9	9
Confirmatory HPLC microcystin analysis	one station every other event	9	9
Total		123	123

[a] Total number of samples for microcystin, chl-*a*/pheo-*a* and quantitative phytoplankton analysis collected at routine stations. Additional samples may be collected as per item #2 and #3 in Section 2.1, Approach. Up to 10 samples per bloom event may be collected and evaluated, with the possibility that additional samples beyond these 10 may be requested .

2.2 Timeline

As noted, initial routine sampling and analysis of microcystins by ELISA was implemented in spring, 2002. The more focused approach detailed in this SAP will be implemented in May, 2003. At a minimum, sampling and analysis will continue through November 2004.

2.3 Sampling Procedures

Protocols for the sampling and analysis of microcystins do not currently exist. However, a working group of the International Organization for Standardization is currently developing such protocols (Chorus, personal communication, April 24, 2002). The following sampling procedures are based on methods of Carmichael (2001), Chorus (2001), Johnston and Jacoby (in press).

Table 2. Sample Container & Preservation Requirements

Parameter	Matrix	Container	Preservation	Hold time
Quantitative or Qualitative Phytoplankton	Liquid	60-mL G	Lugol's solution	365 days
Biovolumes	Liquid	500-mL PP (sterile)	4°C	24 hours
Chlorophyll- *a* (in Lab)	Filtered Liquid	1-L HDPE, AWM15-nl Glass centrifuge tube	4°C90% Acetone, -20°C	28 Days1 day for filtration 28 days for analysis
Pheophytin- *a* (in lab	Liquid	1-L HDPE, AWM (same bottle as collected for lab analysis of chlorophyll- *a*)	4°C	1 day for filtration 28 days for analysis
Microcystins ELISA (MLR-ELISA)	Liquid	500- mlL Amber Glass (for sonication) and one 40-mL VOA vial	4°C	7 Days
Microcystins PPIA (MLR-PPIA)	Liquid	500- mlL Amber Glass (for sonication)	4°C	7 Days
Microcystins HPLC	Liquid	1-L amber glass	4°C	ASAP

Notes:
AWM – Amber wide mouth bottle.
G – Glass bottle
HDPE – High density polyethylene bottle
PP – Polypropylene
VOA – Volatile organics analysis

2.3.1 Water sample collection and storage procedure to test for toxins:

Collect a sample of ambient phytoplankton density specifically targeting cyanobacterial accumulations in the vicinity (grab sample using a 500-mL glass bottle, leaving headspace for freezing). The sample bottle should not be pre-rinsed with sample. Fill

bottle with surface water by keeping the top half of the bottle mouth above the surface during sampling. Do not overfill. A 40-mL VOA vial will also be collected at each station.

- Label the bottles
- Place the sample bottles in a cooler with ice packs (no preservative required).
- Freeze the 500-mL glass bottle and the VOA vial within 2 hours of arrival at the King County Environmental Laboratory. Bottles and vials should be slanted to prevent breakage during freezing. Samples must be stored frozen for a minimum of 12 hours to insure complete freezing of the sample.
- Periodically, one additional 1-L amber glass bottle will be collected for confirmatory HPLC analysis of microcystins. This bottle will be kept at 4˚C and delivered ASAP to the subcontracted laboratory for analysis.

2.3.2 Water sample collection and storage procedure for quantitative identification of cyanobacteria.

At the routine monitoring stations, samples will be collected as part of the Major Lakes Program and analyzed for chl-*a*, pheo-*a*, and qualitative and quantitative cyanobacteria identification and enumeration. See the Major Lakes Monitoring Program SAP and SWAMP Quantitative Phytoplankton Study SAP (King County DNRP 2003) for further information.

In the event that samples are collected as per #2 or #3 in Section 2.1, additional sample volume will need to be collected for cyanobacteria identification. An additional 60 mL aliquot will be collected and placed in properly labeled opaque bottles (typically 60 mL glass vials wrapped in aluminum foil) and preserved with a sufficient amount of concentrated Lugol's solution to turn the sample light red; typically eight drops. Care should be taken that samples are covered tightly and stored in the dark until analyzed.

2.3.3 Water sample collection and storage procedure for chlorophyll *a*/pheophytin *a* analysis.

At the routine monitoring stations, samples will be collected as part of the Major Lakes Program and analyzed for chl-*a*, pheo-*a*, and qualitative and quantitative cyanobacteria identification and enumeration. See the Major Lakes Monitoring Program SAP and SWAMP Quantitative Phytoplankton Study SAP (King County DNRP 2003) for further information.

In the event that samples are collected as per #2 or #3 in Section 2.1, additional sample will need to be collected and preserved for chl-*a*/pheo-*a* analysis. Samples should be stored in the dark at 4°C before filtration, which should take place ASAP and up to 1 day following collection. Filters are then stored in 90% acetone, in a foil-covered rack in a -

20°C freezer (non frost-free) for up to 28 days prior to sonication and instrumental analysis. Once samples are filtered, it is preferred to store the samples on filters for at least two days prior to sonication and analysis to help facilitate extraction of chlorophyll from algae into the acetone medium.

2.4 Sample Preparation for Toxin Assay

To measure total microcystin concentrations (extra- and intracellular) in the water samples, sample preparation will include a cell-lysing step prior to analysis. Established protocols are unavailable at this time, therefore two (2) techniques will be evaluated for effectiveness in lysing. (Note, ELISA measures only free microcystin, not the amount chemically bound to the cell or molecular components such as protein phosphatase enzymes). Cell lysing will be accomplished by:

- Sonication (ultrasonic disruption) using a Vibra Cell Sonicator.
- Freezing of samples for a minimum of 12 hours prior to analysis.

Note unfrozen but refrigerated controls will also be analyzed, to provide data to evaluate the two options listed above. One (1) technique will be recommended to the Study Project Manager following a two to three month evaluation period at the beginning of the program.

The objective of the cell-lysing is to generate a sample in which all microcystins (extra and intracellular) have been converted into a free form which can be measured by ELISA and PPIA, thus providing a close approximation of the total concentration in the ambient sample (extra and intracellular). The resulting concentration should be representative of a recreational exposure in which a swimmer ingests ambient water and cells as a combined dose. Samples analyzed without lysing will be reported as *Free Microcystins*, samples analyzed following lysing will be reported as *Total Microcystins*.

Holding times for microcystin analysis in frozen samples have not been established to date. Other studies have shown that microcystins do not readily degrade in frozen samples (Chorus, personal communication, April 24, 2002). Deep-freezing samples that have been freeze-dried will ensure sample preservation; however, even wet-frozen samples demonstrate no substantial loss in microcystin concentration over months or years. Storage of dried samples at air temperature should be avoided because absorbed moisture from the air may activate the bacteria (Chorus, personal communication, April 24, 2002). Based on KCEL SOP(s) 04-02-009 and 012, a conservative holding time of 7 days will be employed.

3 Laboratory Analysis

The enzyme-linked immunosorbent assay (ELISA) and the protein phosphatase inhibition assay (PPIA) are suitable for rapid and sensitive detection of microcystins. These methods are useful for preliminary toxin screening for both cyanobacterial samples and

extracellular microcystins in the water (Chu *et* al. 1990; Chorus 2001). ELISA is based on the structure of the microcystin molecule and requires antibodies against microcystins whereas PPIA is based on the toxic effects of microcystins. The PPIA method is preferred for waters that may contain toxic forms of microcystins and nodularins.

ELISA and PPIA are suitable as indicating tests for the analysis of extracellular microcystins at concentrations below 1 µg/ L. ELISA is the most sensitive and simple method, but has the potential for false positive reactions (Chorus 2001). PPIA provides preliminary information on the toxicity of microcystins in comparison to the microcystin content measured by ELISA. For confirmation of microcystin, high performance liquid chromatography (HPLC) is recommended (Chorus 2001).

The King County Environmental Laboratory has developed Standard Operating Procedures (SOP) for the measurement of microcystins using ELISA (SOP 04-02-009) and microcystins and nodularins using PPIA (SOP 04-02-012) in water.

3.1 Toxin Structure and Cross-Reactivity Analysis Summary

Microcystins are a group of cyclic heptapeptide hepatotoxins produced by species of the common bloom-forming genera of cyanobacteria including *Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria*. These toxins contain two variable L-amino acids, three D-amino acids and two unusual amino acids. There are now over 50 different microcystins which have been structurally characterized and which differ primarily in the two L-amino acids and methylation or demethylation of the two unusual amino acids. These microcystins all contain the Adda amino acid, which is essential for expression of their biological activity. Nodularins are monocyclic pentapeptide liver toxins produced by the cyanobacterium *Nodularia*. Nodularins contain Adda but lack one of the L- and D-amino acids found in microcystins. Both microcystins and nodularin have been found to be potent inhibitors of protein phosphatase (PP) isozyme types 1 and 2A. The inhibitory action of the toxins on PP1 is considered a basis for their toxicity and forms the basis for the PP1 inhibition assay (Section 1.4.2). Currently several methods have been developed to detect and quantify cyanotoxins. However, there is no single method that provides adequate monitoring for all cyanotoxins. Many of the microcystins and nodularins in environmental samples will be detected by a combination of the following two methods, ELISA and PPIA, as summarized in the following table.

Table 3. Toxins measured by ELISA and PPIA methods.

Toxin	ELISA	PPIA
[DMAdda^5] MC-LR	no	yes
[DMAdda^3] NODLN	no	yes
[6(z)Adda^3] NODLN	no	no
[6(z)Adda^5] MC-LR	-	no
(D-Glu-$OCH_3{}^6$] MC-LR	yes	no

3.1.1 Microcystins– ELISA

The ELISA Kit uses polyclonal antibodies that bind either microcystins or a microcystin-enzyme conjugate. Microcystins in the sample compete with the microcystin-enzyme conjugate for a limited number of antibody binding sites. Since the same number of antibody binding sites are available on every test well, and each test well receives the same number of microcystin-enzyme conjugate molecules, a sample that contains a low concentration of microcystins allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of microcystins allows fewer microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. The plate kit does not differentiate between microcystin-LR and other microcystin variants but detects their presence to differing degrees. At 50% inhibition the concentrations are: MC-LR 0.31 μg/L, MC-RR 0.32 μg/L, MC-YR 0.38 μg/L, and NODLN 0.47 μg/L.

The samples will be filtered through a 0.45 μm filter prior to analysis. **NOTE:** Green pigments and associated substances in 0.45 μm filtrate can mask the presence of microcystins. Filtration to 0.45 μm and then to 5000 nominal molecular weight limit (nmwl) is recommended to remove these pigments and associated substances. Since the ELISA requires 50 μL per replicate, a scaled up version of the ultrafiltration system, perhaps including centrifuge, may be most efficient (see attachment for further discussion). The method detection limit (MDL) is 0.05 μg/L as microcystin-LR equivalents.

3.1.2 Microcystins –PPIA

The enzyme protein phosphatase is inhibited in a concentration-dependent manner by microcystins. Subsequent exposure of the enzyme to a substrate that forms a colored product reveals the degree of enzyme inhibition. Comparison of sample results with those of known standards quantifies the level of microcystins in the sample.

The samples will be prepared by filtration to 0.45μm to remove particulates and to 5000 nmwl to remove compounds that interfere with the assay. The MDL for the PPIA is 0.1 μg/L as microcystin-LR equivalents.

3.1.3 Microcystins - HPLC

A subsample of stations will be submitted to Water Management Laboratories Inc. in Tacoma Washington for confirmation of total microcystins by High Performance Liquid Chromatography (HPLC). The MDL for the HPLC is < 0.1 μg/L as microcystin if provided with 100 mL of sample (personal communication with lab). The number of these confirmatory samples and frequency of collection and analysis by HPLC will be determined by the Project Manager.

3.2 Analytical Procedures

Samples will be analyzed using the procedures and detection limits listed in the table below.

Table 4. Laboratory Analysis Summary

Parameter	Reference	Method Detection Limit	Reporting Detection Limit
Microcystins by ELISA	KCEL SOP 04-02-009	0.05 μg/L	0.05 μg/L
Microcystins and Nodularins by PPIA	KCEL SOP 04-02-012	0.1 μg/L	0.1 μg/L
Confirmatory Microcystins by HPLC	WML Inc;	0.1 μg/L	0.1 μg/L
Chlorophyll *a*	EPA 446.0	0.5 μg/L	1.0 μg/L
Pheophytin *a*	EPA 446.0	1.0 μg/L	2.0 μg/L

4 Data Quality Objectives

The procedures and practices described in this QA plan are designed to generate data of sufficient quality to support decision making as discussed in the site specific SAP. Critical elements of laboratory data quality objectives are discussed in this section. Procedures to attain these data quality objectives are discussed throughout this document. In particular, Section 7.0, Quality Control Procedures, addresses many of the procedures necessary to obtain data that meet these data quality objectives.

4.1.1 Laboratory Precision

Laboratory precision will be assessed using laboratory duplicates. When both sample results are at or exceed the MDL the RPD (relative percent difference) should be less than 25 %. An RPD cannot be determined unless both values are at or above the MDL since no values are reported if <MDL. Note that the Method Detection Limit (MDL) and the Reporting Detection Limit (RDL) are the same for both the ELISA and PPIA.

The actual criteria for performing the RPD calculation and applying the control limits are based on at least one of the values being >RDL. If both results are <RDL, no calculation is applied and there are no expectations placed on the data with respect to precision.

If one value is >RDL and the other <MDL, a RPD is still calculated using zero for the less <MDL value.

A 25% RPD is applicable to chlorophyll but a 50% window is used for pheophytin.

4.1.2 Field Precision

Information regarding the precision of sampling procedures will be obtained by collecting field replicates. The data user should take the information obtained by collecting field replicates into account when making decisions based on data generated under this QA plan.

4.1.3 Bias

Bias is an indicator of the accuracy of analytical data. For this project, laboratory control samples or blank spikes, whichever are available, will be used to assess bias. Results should be within 20% of the true value or within the criteria provided with the purchase of the control sample.

Bias will also be assessed by the evaluation of field blank and method blank data. Analytical results for method blanks should be less than the MDL (method detection limit). Note that some common organics laboratory contaminants may exceed the reported MDL.

The use of matrix spike recovery data will provide additional information regarding method performance on actual samples. The laboratory will use professional judgment regarding assessment of data quality and any subsequent action taken as a result of matrix spike recoveries.

4.1.4 Representativeness

This survey is primarily designed to evaluate the presence/absence of cyanobacterial toxicity, and secondarily to estimate concentrations and geographic extent of the toxin distribution, should it be present. Representative samples will be obtained through the following practices:

- The use of generally accepted sampling procedures will allow for the collection of representative samples.
- Subsampling within the King County Environmental Laboratory will be conducted according to lab standard operating procedures. These procedures are designed to obtain representative subsamples.

Note that additional practices to be used to obtain representative data are described in the site specific SAP. (see Major Lakes Monitoring Program SAP, King County, forthcoming in 2003.)

4.1.5 Comparability

Data comparability will be obtained through the use of standard sampling procedures and analytical methods. Additionally, adherence to the procedures and QC approach contained in this QA Plan will provide for comparable data throughout the duration of this project. Before making changes to sample collection, storage or analysis procedures, each must be evaluated to verify that comparability will not be compromised.

4.1.6 Completeness

Completeness will be evaluated by the following criteria:

- The number of usable data points compared to the projected data points as detailed in this QA plan.
- Compliance with the data quality criteria as presented in this section.
- Compliance with specified holding times.

The goal for the above criteria is to obtain 100% data completeness. However, where data are not complete, decisions regarding re-sampling and/or re-analysis will be made by a collaborative process involving both data users and data generators. These decisions will take into account the project data quality objectives as presented above.

5 Data Reduction, Review, and Reporting

Data reduction, review and reporting will be performed under the King County Environmental Laboratory's standard operating procedures. Laboratory data will be provided to data recipients within 30 days of sample receipt. Data reports will include sufficient information to conduct the data assessment detailed in Section 6.0. Field measurements will also undergo standard reviewing and reporting procedures. Data will be reported in the standard laboratory-reporting format. This includes an analytical result, MDL and RDL, if available. The reporting format and standard due dates for quantitative phytoplankton data will be defined by the contract that King County establishes with Water Environmental Services, Inc.

Protocols will be worked out with the King County Environmental Laboratory for the rapid turn around of selected samples in the event of a bloom episode that could have potential public health implications. Preliminary Project Data, required in the event of a bloom episode that could have potential public health implications, will be reported using KCEL Preliminary Data Reporting Form followed by final data as soon as practical.

Final project data will be presented to the project and program managers in a format that will include the following:

- King County Environmental Laboratory Comprehensive Reports consisting of spreadsheets of analytical and field parameters;
- Case narratives for ELISA and PPIA results prepared by the Aquatic Toxicology Section;
- Section narratives of chemistry and microbiology data including supporting QC documentation (provided by the King County Environmental Laboratory) in the event of analytical or data anomalies.
- A technical memorandum summarizing field sampling, analytical work and interpretation of the QC results (provided by the King County Environmental Laboratory).

- Cyanobacteria identification and biovolume determinations conducted by Water Environmental Services, Inc., as per contract and the Major Lakes Quantitative Phytoplankton SAP (King County DNRP 2003).

6 Project Organization

Project team members and their responsibilities are summarized below. All team members are staff of the King County Department of Natural Resourcesand Parks Water and Land Resources Division. Jean Jacoby, Professor and chair of the Department of Civil and Environmental Engineering at Seattle University, serves as a Technical Consultant to the SWAMP Program for the Cyanobacteria Study.

Table 5. Project Team Members

Name/Telephone	Title	Affiliation	Responsibility
Jonathan Frodge (206) 296-8018	Senior Water Quality Planner	Modeling, Assessment, and Analysis Unit	Program manager for SWAMP.
Curtis DeGasperi (206) 296-8252	Water Quality Engineer	Wastewater Treatment Division	Project manager for the Major Lakes Quantitative Phytoplankton Study.
Katherine Bourbonais (206) 684-2382	Laboratory Project Manager	Environmental Laboratory	Coordination of analytical activities, lab QA/QC and data reporting.
Jeff Droker (206) 684-2309	Environmental Specialist	Environmental Laboratory	Coordination of sampling activities, field QA/QC, and field analyses.
Debra Bouchard (206) 263-6343	Water Quality Planner	Water & Land Resources	Project manager for the Toxic Cyanobacteria Study, coordination between lab, contracted phytoplankton specialist, and in-house specialist
Colin Elliott (206) 684-2343	Quality Assurance Officer	Environmental Laboratory	Overall project QA/QC.
Fran Sweeney (206) 684-2358	Aquatic Toxicologist Supervisor	Environmental Laboratory	Coordination of toxicity analysis
Jim Buckley (206) 684-2314	Aquatic Toxicologist	Environmental Laboratory	ELISA and PPIA method development
Jean Jacoby (206) 296-5526	Professor and Chair Dept of Civil and Environmental Engineering	Seattle University	Technical Consultant to SWAMP Cyanobacteria Study

7 Quality Control Procedures

7.1 Field Quality Control Procedures

Field meters (pH and conductivity) will be calibrated in the field using standard solutions. Field meters will be recalibrated according to current ESS standard operating procedures. For all samples collected in replicate, a duplicate field determination will be made. All calibration and duplicate data will be recorded in the field records.

Over the course of this project, field QC samples will be collected at the frequency listed below. It is recommended that a set of field QC samples be collected during the first sampling effort to provide an initial indication of field sampling precision and bias.

Table 6. Field Quality Control Samples

Type of Quality Control Sample	Description	Frequency
Field Blank	Reagent sample matrix that has been processed as a field sample, used as an indication of sampling process contamination	Over the course of the project, 1 per sampling day, done at a random site. If more than 20 sites are collected in one day, 2 field blanks should be collected.
Field Replicate	A second sample generated from the same sampling location as the initial sample, but from a second sampler deployment. Used as an indicator of field sampling precision.	Over the course of the project, 1 per sampling day, done at a random site. If more than 20 sites are done in a day, 2 field replicates should be collected.

7.1.1 QC Practices for Field Measurements

Sampling for this Toxic Cyanobacteria Study is conducted concurrently with the Routine/Ambient Major Lakes Monitoring program. Therefore QA practices are covered under those SAPs.

7.2 Laboratory Quality Control Procedures

The King County Environmental Laboratory is accredited by the Washington State Department of Ecology. As a requirement of this accreditation, the lab is audited by the Washington State Department of Ecology. Additionally, the King County Environmental Laboratory participates regularly in US EPA inter-laboratory performance evaluation studies.

A number of samples will also be analyzed by HPLC for microcystins. Both ELISA and PPIA are suitable as indicating tests for the analysis of extracellular microcystins, but ELISA has potential for false positives. Therefore, confirmatory analysis using a different determinative approach will provide information that can be used to evaluate ELISA data. The number and frequency of confirmatory sample analyses will be determined by the Project Manager.

7.2.1 Frequency of quality control samples

For samples analyzed at the King County Environmental Laboratory, the frequency of quality control samples to be performed for this project is shown in the following table. QC samples shown below may not be available for all lab analysis.

Table 7. Laboratory Quality Control Samples

Type of Quality Control Sample	Description	Frequency
Method Blank	An aliquot of clean reference matrix carried through the analytical process and used as an indicator of contamination.	1 per sample batch. Maximum sample batch size equals 20 samples.
Laboratory Control Sample	Solution of known analyte concentration, processed through the entire analytical procedure and used as an indicator of method accuracy and precision	1 per sample batch. Maximum sample batch size equals 20 samples, as available.
Spike Blank	Known concentration of target analyte(s) introduced to clean reference matrix, processed through the entire analytical procedure and used as an indicator of method performance.	Used if a laboratory control sample is not available 1 per sample batch. Maximum sample batch size equals 20 samples.

In addition to the QC samples specified above, the following QC samples will be performed on samples from this project at the frequency listed below:

Table 8. Additional Laboratory Quality Control Samples

Type of Quality Control Sample	Description	Frequency
Lab Duplicate	A second aliquot of a sample, processed concurrently and identically with the initial sample, used as an indicator of method precision.	Over the course of the project, 1 per 20 samples.
Matrix Spike	An aliquot of sample to which known quantities of analyte(s) are added. Used as an indicator of sample matrix effect on recovery of target analyte(s).	Over the course of the project, 1 per 20 samples.
Matrix Spike Duplicate	An additional matrix spike sample used as an indicator of matrix effect on sample recovery and method precision.	May be analyzed instead of a sample duplicate.

KCEL laboratory QC samples for chl–*a*/pheo-*a* and microcystins analysis and associated control limits are summarized below. These QC samples will be analyzed at a frequency of one per analytical batch 20 or fewer samples.

Table 9. Laboratory QC Requirements

Parameter	Method Blank	Duplicate RPD	Negative Control	CS % Recovery
Chl-*a*	<MDL	25%	NA	90 to 110 %
Pheo-*a*	<MDL	50%	NA	NA
Microcystins	<MDL		<0.1 ppb	NA

Notes:
CS- Check Standard (positive control equivalent Laboratory Control Sample)
MDL – Method Detection Limit
NA – Not Applicable
RPD – Relative Percent Difference

7.3 Corrective Action

King County Environmental Laboratory standard operating practice is to detect and correct analytical difficulties during sample analysis. Should the lab have difficulty in meeting the data quality objectives outlined in this QA plan, the lab will work with the data user to develop and implement corrective action.

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9 Attachment –THE INFLUENCE OF PIGMENTS ON RESULTS IN THE PROTEIN PHOSPHATASE INHIBITION ASSAY

Lab Analysis conducted by Jim Buckley

020123 JB

In the ELISA for microcystins, it is not uncommon to observe anomalous %B values of greater than 100% for environmental samples. The same is true for the PPIA. When the samples from the *Aphanizomenon* bloom in Green Lake were analyzed by PPIA, samples filtered to 0.45 um retained a green color and yielded %B values much greater than 100%. This provided an opportunity to test for pigment removal with an ultrafiltration system consisting of a filter of 5000 nmwl and centrifugation at 15,000 g.

The following table shows results of three days of tests of several treatments of Green Lake SE (GL SE) and Aquatheater (GL AT) samples designed to identify the influence of pigments, probably mostly chlorophylls, on the results of PPIA. Values in bold print are from samples that were green in color following filtration to 0.45um.

Terms:
0.45 = filtration to 0.45 um with glass fiber prefilter and 0.45 um Millipore Millex filter
5000 = filtration to 5000 nominal molecular weight limit (nmwl) Millipore Ultrafree centrifuge unit
control = assay mixture including sample but without PP enzyme
blank = negative control without PP enzyme
centrifuge = 10 min. at 15,000g

Sample /Treatment	GL SE 1		GL SE 2		GL AT 1		GL AT 2	
	%B	ug/L	%B	ug/L	%B	ug/L	%B	ug/L
12-29-01								
0.45 + 5000	90	0.095	92	0.087	90	0.093	86	0.111
control	2		-15		3		4	
blank = 0.069								
1-8-02								
0.45	**373**	**0.000**	**442**	**0.000**	95	0.082	92	0.092
0.45 + 5000	94	0.085	95	0.081	91	0.094	91	0.095
control	-0.1		-1.0		-1.0		-4	
blank = 0.107								
1-10-02								
0.45	**391**		**489**					
control	**285**		**376**					
0.45 + centrifuge	**355**		**421**					
control	**257**		**332**					
blank = 0.075								

12-29-01
The samples were filtered to 0.45 (green) and then to 5000 (clear) and assayed for microcystins. Results showed microcystins present at 0.111 ug/L in the GL AT 2 sample. All other samples gave results < MDL of 0.1 µg/L. Control %B values were ≤ 4, indicating little or no apparent endogenous PP activity.

1-8-02
Samples from GL SE 1 and 2 filtered to 0.45 um only gave very high %B values (**373** and **442**) due to the OD from the green pigments and perhaps from other substances also. In comparison, samples GL AT 1

and 2, also filtered to 0.45 um, were clear and gave typical %B values (95 and 92). When the 0.45-filtered GL SE 1 and 2 samples were further treated by filtration to 5000 nmwl thereby removing the green color and putative other substances, the %B values were reduced from **373** to 94 and **442** to 95. This indicates that the 5000 nmwl filtration step is effective in removal of pigments and other substances that can confound results of the PPIA. Filtration of GL AT 1 and 2 to 5000 nmwl only slightly changed the % B values (95 to 91 and 92 to 91) and resulting levels of microcystins. Control values for these samples were all very low (-0.1 to -1.0).

1-10-02
Samples were green following filtration to 0.45 um only. Subtraction of the control (without PP) from the assay (with PP) yields values (mean = 102) that are close to the negative control (with PP) indicating little or no endogenous PP activity in these samples.

GL SE 1: **391** – **285** = 106 A % B value of ≈ 100 indicates a sample reading ≈ negative control

355 – **257** = 98

GL SE 2: **489** – **376** = 113

421 – **332** = 89

Centrifugation alone yielded a small white button for both samples. However, the high %B values were only reduced by 9 to 14 %, indicating that only a small amount of the high OD is due to particulates.

GL SE 1: **391** – **355** = 36 or 9% of %B is removable by centrifugation

GL SE 2: **489** – **421** = 68 or 14% of %B is removable by centrifugation

Conclusions

1. Green pigments and associated substances in 0.45 um filtrate give artificially high %B values that can mask the presence of microcystins.

2. Filtration to 0.45 um and then to 5000 nmwl removed these pigments and associated substances that, in this case, gave high %B values that could mask otherwise detectable levels of microcystins.

3. It is useful to run Controls to check for apparent endogenous protein phosphatase activity in samples. In the present samples, there was no apparent protein phosphatase activity.

4. Centrifugation only of samples previously filtered to 0.45 um showed that 9 to 14 % of the high %B values was due to particulates amenable to removable by filtration.

5. For the Green Lake SE samples, the yield from the ultrafiltration system was 120 uL each which was adequate for the PPIA which requires 20 uL per replicate. To be useful for the ELISA which requires 100 uL per replicate, a scaled up version of the ultrafiltration system, perhaps including centrifuge, would be most efficient.