SAMPLING AND ANALYSIS PLAN – 2007
PHASE III FOR TOXIC CYANOBACTERIA IN LAKE WASHINGTON, LAKE SAMMAMISH, AND LAKE UNION

SUBMITTED TO:

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NAME OF PROJECT: Sampling and Analysis Plan for Toxic Cyanobacteria in Lake Washington, Lake Sammamish and Lake Union.

Project Number: **421235 – Routine Major Lakes Sampling and Analysis**

421395 – SWIMMING BEACHES SAMPLING AND ANALYSIS

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1 Study Design

Changes to the Phase II Toxic Cyanobacteria study design were implemented in 2006 and are noted below. These modifications were made in an effort to reduce spending on Quantitative Phytoplankton analysis. The basic study design for 2007 will remain the same as during 2006. Note an additional swimming beach location, A734WSB, west Green Lake, was added to the program as of May 31, 2007. Samples will be collected at this location during the remaining 9 events of the 2007 season.

1.1 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). The monitoring efforts described in this Phase III Sampling and Analysis Plan (SAP) Addendum will begin in March 2007. After each year of monitoring microcystins, the monitoring program will be re-evaluated and the sampling design optimized. At that time, the SAP will be revised or amended as needed. **NOTE**: the onset of this monitoring effort will cancel the previously ongoing cyanobacteria toxicity testing at Routine Major Lakes Sites.

Sample collection will utilize the combined efforts of the Routine Major Lakes Sampling Program and the Swimming Beach Monitoring Program. Table 1 lists the specific sampling sites for microcystin analysis. Spreadsheet A, the 2007 Cyanobacteria Sampling Schedule (attached), lists all Major Lake sampling sites and swimming beach sites included in this study, and illustrates how microcystin and quantitative phytoplankton sample collection is coordinated with these programs. The Major Lakes Sampling Program collects samples twice per month from March through October. Swimming Beach Monitoring occurs weekly from mid-May through mid-September, with samples typically collected on Tuesdays. Coordination with both programs will provide for weekly sample collection throughout most of the productive growing season and for better tracking of microcystin production in the lakes.

An example of the May through September monthly sampling scenario is as follows:

- Week 1 10 Swimming Beach sampling sites
- Week 2 13 Routine Major Lake sampling sites
- Week 3 10 Swimming Beach sampling sites
- Week 4 13 Routine Major Lake sampling sites, etc.

During the months of March, April and October, when the Swimming Beach Monitoring is not taking place, sampling will occur at the Routine Major Lakes sites only. During months when there are 3 weeks between lake sampling, samples will be collected at the Swim Beach sites.

Table 1. Summary of Cyanobacteria Toxicity Sampling Design.

Routine Major Lake Sampling Site	Samples per visit	Visits per year (March - October)	**Swim Beach Sampling Site**	Samples per visit	Visits per year (mid-May-mid-Oct)
0807	1	16	0806SB – Juanita	1	10
0826[a]	1	16	0826SB – Magnuson	1	10
4903	1	16	4903SB – Pritchard Is.	1	10
0834	1	16	0834SB – Meydenbauer	1	10
0832	1	16	083930SB – Newcastle	1	10
0831 [a]	1	16	0828SB – Gene Coulon	1	10
0852 [a, c]	1	16	0852SB – Madison	1	10
0817	1	16	0818SB - Matthews	1	10
0625	1	16	0602SB – Idylwood	1	10
0611 [a]	1	16			
0614	1	16	0615SB –Lake Samm State Park	1	10
0612 [a, c]	1	16	---	---	---
A522 [a, c]	1	16	A734WSB – west Green Lake	1	9
Field Replicate (one station every other event)					
0852 [a]	alternate visits	8	0806SB	alternate visits	5
TOTAL [b]		216			114

a. Indicates integrated composite sample. All other samples are discrete surface grabs. See Section 2.3.1.

b. Total number of routinely collected samples for microcystin analysis. Chlorophyll-*a* (chl-*a)* and pheophytin-*a*(pheo-*a)* analysis will be carried out at Routine Major Lake Stations during all sampling events.

C. Quantitative phytoplankton samples will be collected and analyzed from 0852, 0612, and A522 during each sampling event included in this study, and once per month during November through February. In addition, quantitative phytoplankton samples will be collected from all other Routine Major Lakes stations and swimming beach sites listed in Table 1 above and Spreadsheet A (attached). These quantitative phytoplankton samples will be archived for possible future analysis as pending results of the microcystin analysis. One sample per site may be collected per bloom event and evaluated, with the possibility that additional samples may be requested, as per items #2 and #3, Section 2.1.

The following three types of sampling scenarios are included in this study:

1. Routine Major Lakes Sampling. Thirteen sampling sites were selected at Routine Major Lake monitoring locations in order to relate cyanobacterial data to other lake data. At least one deep station is included in each lake and the rest are nearshore sites that are within close proximity to selected swimming beaches monitored by the County (Table 1 and Spreadsheet A).

   An aliquot of the sample collected as part of the routine sampling effort will be used for this study. Sample collection in the Routine Major Lakes program has been modified to incorporate use of one of two methods – either an integrated composite sample, or a discrete surface sample. Spreadsheet A identifies which sampling technique is used at each site in the overall Major Lakes Program. Section 2.3.1 describes the two sample collection methods.

   Microcystin will be measured by ELISA and PPIA using the extraction methods described in the Phase II SAP. Chl-*a*/pheo-*a* analysis will be conducted on all thirteen of the Major Lakes samples as part of this Routine Major Lakes Monitoring effort. See Major Lakes Monitoring Program SAP for further discussion. **NOTE** that quantitative phytoplankton enumeration and identification is being performed routinely for three samples collected from the Major Lake stations 0852, 0612, and A522 as part of this focused Toxic Cyanobacteria Study (Table 1 and Spreadsheet A). Phytoplankton samples will be collected at the other Major Lake stations noted in the table above, and archived for future identification and enumeration as determined necessary by microcystin and/or chl-*a* results.

2. Swimming Beach Monitoring. The second component of this sampling scenario will be conducted by the King County Environmental Laboratory's (KCEL's) Environmental Services Section (ESS) staff as part of the Swimming Beaches Monitoring Program. Ten stations that are part of the seasonal monitoring for fecal coliform bacteria will be included in this round of the focused Toxic Cyanobacteria Study (Table 1 and Spreadsheet A).

   Sufficient sample volume will be collected for microcystin testing and quantitative phytoplankton identification and enumeration. Quantitative phytoplankton identification and enumeration samples will be archived and analyzed if determined necessary by high microcystin concentrations. If toxins are present, quantitative phytoplankton identification and enumeration may be determined using the same methodology as for the Routine Major Lakes sampling effort. Sample collection will be a surface dip.

   In addition, ESS will routinely visually inspect the waters at other swimming beach stations for cyanobacteria blooms while conducting the Swimming Beaches program. One sample per site may be collected per bloom event, followed by Project Manager evaluation, and subsequent decisions regarding appropriate next steps.

3. 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 lakes 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. Coordinates will be obtained for these grab samples and a LIMS locator created. New locator names will be consistent with the naming convention system established for the Major Lakes Program. One sample per site 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, as well as chl *a*/pheo-*a*, and phytoplankton quantitative enumeration and identification, if necessary. Microcystin will be measured by ELISA and PPIA on these discrete samples using the extraction methods described in the Phase II SAP. If toxins are present, chl–*a*/pheo-*a* and quantitative phytoplankton identification and enumeration may be determined using the same methodology as for the Routine Major Lakes sampling effort. See Major Lakes Monitoring Program SAP for further discussion.

1.2 Timeline

As noted, initial routine sampling and analysis of microcystins by ELISA was implemented in spring, 2002. This was followed by the more focused approach (detailed in the previous Cyanotoxicity SAP) from May 2003 and through November 2004. The Phase II SAP was implemented during March 2005 and continued through October 2006. Modifications as outlined in the 2006 Phase II addendum were implemented in March, 2006. Those modifications and others outlined in this Phase III SAP Addendum will be implemented in March, 2007.

1.3 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 (2002).

Table 2. Sample Container & Preservation Requirements

Parameter	Matrix	Container	Preservation	Hold time
Quantitative Phytoplankton	Liquid	•1x 60-mL Glass wrapped in foil	•Lugol's solution, added in the field, store in the dark	365 Days
Quantitative Phytoplankton	Liquid	•1x 250 ml Plastic Amber Wide Mouth (AWM)	•Lugol's solution added in the field •Store at room temperature in the dark	•365 days
Chlorophyll- *a* (in lab) (CHLA)	Liquid	1-L HDPE, AWM	4°C	1 day for filtration 28 days for analysis
Pheophytin- *a* (in lab) (PHEO)	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	250- ml Glass, AWM wide mouth	4°C	24 to 48 hours then freeze
Microcystins PPIA (MLR-PPIA)	Liquid	250- ml Glass, AWM wide mouth (same bottle as collected for MLR-ELISA)	4°C	24 to 48 hours then freeze

Notes:
AWM – Amber wide mouth bottle
HDPE – High density polyethylene bottle

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

Samples will be collected using the site-specific collection method identified above in Section 2.1 (e.g., integrated composite, discrete surface, or surface grab).

Integrated Composite technique: Vertically integrated composite samples are collected using a weighted length of ¾-inch tygon tubing let down vertically through the water column as done for the Routine Major Lakes sampling program. This tube is marked so that when fully extended, the distance from the mark at the water surface to the end of the tube is 10 m. The tube is plugged at the submerged end by a check valve and retrieved. The tube contains a vertically integrated sample of the lake from surface to 10 meters. The sample is decanted into a stainless steel bowl and homogenized before sub-sampling for microcystin, chl-*a*, pheo-*a* and phytoplankton enumeration. If more than one tube is collected, combine the water in the steel bowl prior to filling sample containers. Aliquots for microcystin analysis will be poured into a 250-mL glass AWM bottle, leaving some headspace for freezing. The sample bottle should not be pre-rinsed with sample.

Discrete Surface Samples: Discrete surface samples are grab samples collected 1 m below the water surface using Scott bottles or Niskin bottles on the CTD rosette.

Swimming Beach surface grabs: For surface grabs, fill the 250-mL glass, AWM bottle by dipping the bottle mouth-down into the water. With a sweeping arch, collect water from approximately 2 feet below the surface, leaving a headspace.

- Label the bottles if not pre-labeled.
- Place the sample bottles in a cooler with ice packs (no preservative required).
- Subsamples will be removed from the 250-mL glass bottle and frozen within 24 to 48 hours of arrival at the KCEL. 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.

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

Quantitative cyanobacteria identification and enumeration will be conducted routinely at the three Major Lake stations as part of Major Lakes Routine Monitoring Program (Table 1 and Spreadsheet A). Samples collected in 2006 were subcontracted to Maribeth Gibbons at WATER Environmental. The KCEL is currently working on method development for quantitative phytoplankton identification and enumeration. Until this method development is complete, samples collected during 2007 will also be subcontracted to WATER Environmental. Additional quantitative phytoplankton identification and enumeration samples may be subcontracted if high values of microcystins warrant further investigation. Samples subcontracted to WATER Environmental will be paid for by the KCEL through the existing purchase order set up by the Project Manager (B17570B).

In addition, samples for quantitative identification and enumeration will be collected and preserved at the designated Major Lakes and Swimming Beach sites in the event that high microcystin or chl-*a* concentrations warrant further investigation. A 250 mL aliquot will be collected and placed in properly labeled opaque bottles (typically, 250 mL AWM plastic container) and preserved with a sufficient amount of concentrated Lugol's

solution to turn the sample light red; approximately 1.75 mL. Care should be taken that samples are covered tightly and stored in the dark until analyzed. This container will be delivered by the sample manager to microbiology staff for appropriate preservation and storage.

All containers will be stored at the laboratory for one month pending availability of microcystin and chl-*a* results. These results will be used when they become available to screen samples to be archived for possible future quantitative identification. The Project Manager will complete this review within 14 days of final data posting to LIMS. Screening criteria include:

- any >MDL detection of microcystins by ELISA or PPIA; or
- chl-*a* concentration 5.0 mg/L or greater

Samples may be disposed of after the review period is over if they do not meet these criteria and the Project Manager has not requested that selected samples be archived. Samples collected at swimming beach locations, which are not routinely analyzed for chl-*a*, will be evaluated on the basis of chl-*a* values for samples collected from nearby lakes locations.

In the event that algal blooms are sampled (as per #3 in section 1.1 above), samples will be collected and preserved as described above.

At the end of the 2007 study season (October 31, 2007), the Project Manager will review samples that have been archived and decide which, if any, should be submitted for quantitative cyanobacteria identification. Samples not submitted for further analysis will be disposed of or archived at an alternative, non-laboratory location.

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

Samples are collected for chlorophyll *a*/pheophytin *a* analysis as part of the Major Lakes Program using either the integrated composite sampling or discrete surface sampling method identified for each site in Spreadsheet A. In the event that algal bloom samples are collected as per #3 in Section 1.1, additional sample volume will need to be collected and preserved for possible chl-*a*/pheo-*a* analysis.

In general, 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.

See the Major Lakes Monitoring Program SAP for more details (King County 2005).

2 Laboratory Analysis

ELISA and PPIA assays are suitable for rapid and sensitive detection of microcystins. These methods are useful for preliminary toxin screening for both cyanobacterial samples and extra-cellular 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 extra cellular 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 high microcystin concentrations, HPLC analysis is recommended (Chorus 2001).

The KCEL 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.

2.1 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. 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 ELISA and PPIA methods.

2.1.1 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.

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 that 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. If samples were analyzed without lysing, results would be reported as *Free Microcystins.* Since all samples collected for this study will be analyzed following lysing, results are equivalent to *Total Microcystins*. Note ELISA measures only free microcystin, not the amount chemically bound to the cell or molecular components such as protein phosphatase enzymes.

Established protocols for extraction are unavailable at this time. The 2002 - 2004 focused cyanobacteria toxin study utilized two techniques to evaluate their effectiveness in lysing – 1) freezing of samples for a minimum of 12 hours, or 2) sonication. Unfrozen but refrigerated controls were also analyzed, to provide data to evaluate the two options listed above. Evaluation of the two extraction methods was inconclusive due to low microcystin concentrations. For this 2005 – 2006 round of sampling, laboratory staff recommended combining extraction methods. Therefore, each sample will receive the following lysing process:

- 10-ml aliquots will be frozen for a minimum of 12 hours and then
- thawed at room temperature and then immediately sonicated (ultrasonic disruption) using the Vibra Cell Sonicator.
- 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. Additional filtration to 5000 NMWL will be performed when the filtrate appears colored to remove pigments and associated substances that may interfere with the assay. Since the ELISA requires 50 μL per replicate, a scaled up version of the ultra filtration 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. MDL for the PPIA is 0.1 μg/L as microcystin-LR equivalents.

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 for frozen samples of 7 days will be employed. Holding times for the filtrate at 4 ºC are being determined.

2.1.2 Microcystins– ELISA

The ELISA test 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 is 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.

2.1.3 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.

2.2 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
Quantitative Phytoplankton Identification	KCEL SOP draft, in review	NA	NA
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
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

2.2.1 Laboratory Precision

Laboratory precision for chemical analyses will be assessed using laboratory duplicate (LD) or matrix spike / matrix spike duplicate (MS / MSD) QC samples. 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.

See Table 8 for RPD control limit windows.

2.2.2 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 SAP.

2.2.3 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. See Table 8 for percent recovery control limit windows.

Bias will also be assessed by the evaluation of method blank data. Analytical results for method blanks should be less than the 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.

2.2.4 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 KCEL 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; Major Lakes Monitoring Program SAP, King County, 2005.

2.2.5 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 SAP 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.

2.2.6 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 SAP.
- 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.

3 Data Reduction, Review, and Reporting

Data reduction, review and reporting will be performed under the KCEL's standard operating procedures. Laboratory data will be available electronically to data recipients within 30 days of sample receipt except for quantitative phytoplankton identification results. These results will be reported on or before December 31, 2007, for all samples collected during 2007. Hard-copy data reports, if requested, will include sufficient information to conduct data assessment. Field measurements will also undergo standard review and reporting procedures, and 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 subcontracted quantitative phytoplankton data will be defined by the contract that King County establishes with Water Environmental Services, Inc.

Protocols will be established with the KCEL 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:

- KCEL Comprehensive Reports consisting of spreadsheets of analytical and field parameters, if requested;
- Case narratives for ELISA and PPIA results prepared by the Aquatic Toxicology unit;
- Unit narratives of chemistry and microbiology data including supporting QC documentation (provided by the KCEL) in the event of analytical or data anomalies.
- A narrative summary of field and analytical QC results (provided by the KCEL) if requested.
- Cyanobacteria identification and biovolume determinations conducted by KCEL.
- Cyanobacteria identification and biovolume determinations conducted by Water Environmental Services, Inc., as per contract and the Major Lakes Quantitative Phytoplankton SAP (King County DNRP 2005).

4 Project Organization

Project team members and their responsibilities are summarized below. All team members are staff of the King County Department of Natural Resources and Parks, Water and Land Resources Division.

Table 5. Project Team Members

Name/Telephone	Title	Affiliation	Responsibility
Katherine Bourbonais (206) 684-2382	Laboratory Project Manager	Environmental Laboratory	Coordination of analytical activities, lab QA/QC and data reporting.
David Robinson (206) 684-2329	Environmental Scientist	Environmental Laboratory, ESS	Coordination of lake sampling activities, field QA/QC and field analyses.
Judy Ochs (206) 684-2347	Environmental Scientist	Environmental Laboratory, ESS	Coordination of swimming beach 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.
Gabriela Hannach (206) 684-2301	Environmental Scientist	Environmental Laboratory, Aquatic Toxicology	Coordination of toxicity analysis
Jim Buckley (206) 684-2314	Environmental Scientist	Environmental Laboratory, Aquatic Toxicology	ELISA and PPIA method development
Karl Bruun (206) 684-2378	Environmental Scientist	Environmental Laboratory, Microbiology	Quantitative phytoplankton method development
Duc Nguyen (206) 684-2377	Environmental Scientist	Environmental Laboratory, Conventionals	Coordination of chl-*a*/pheo-*a* analysis

5 Quality Control Procedures

5.1 Field Quality Control Procedures

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 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 study, every other sampling event, done at a predetermined site; 0852 (Major Lakes) and 0806SB (Swimming Beaches).

5.1.1 QC Practices for Field Measurements

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

5.2 Laboratory Quality Control Procedures

The KCEL 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.

5.2.1 Frequency of quality control samples

For samples analyzed at the KCEL, the frequency of quality control samples to be performed for this project is shown in the following table. All listed types of QC samples shown below may not be available for all lab analyses.

Table 7. Laboratory Quality Control Samples

Type of Quality Control Sample	Description	Frequency
Method Blank	An aliquot of clean reference matrix carried through the	1 per sample batch. Maximum sample batch

	analytical process and used as an indicator of contamination.	size equals 20 samples.
Laboratory Control Sample (LCS)	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, as available. Maximum sample batch size equals 20 samples.
Check Standard (CS)	A solution of known analyte concentration(s) that is prepared independently from calibration standard solutions, and analyzed along with the samples in the analysis sequence; used to check accuracy of the calibration and indicate between-batch precision.	1 per sample batch, as available. Maximum sample batch size equals 20 samples.
Negative Control (NC)	A solution obtained from the ELISA kit manufacturer with a confirmed microcystin concentration of zero.	1 per sample batch. Maximum sample batch size equals 20 samples.
Matrix Spike/ Matrix Spike Duplicate (MS / MSD)	A MS is a second sample aliquot fortified with a known concentration of target analyte(s), and processed through the entire analytical procedure; used as an indicator of sample matrix effect on the recovery of target analyte(s). A MSD is third sample aliquot fortified with a known concentration of target analyte(s), and processed through the entire analytical procedure; used as an indicator of sample matrix effect on the recovery of target analyte(s) as well as method precision. MS / MSD used with methods where samples typically show no detectable responses, thus do not provide useful information of batch precision.	1 per sample batch. Maximum sample batch size equals 20 samples.
Spike Blank (SB)	Known concentration of target analyte(s) introduced to clean reference matrix, processed	Used if a laboratory control sample is not available.

	through the entire analytical procedure and used as an indicator of method performance.	1 per sample batch. Maximum sample batch size equals 20 samples.
Lab Duplicate	A second aliquot of a given sample, processed concurrently and identically with the initial sample, used as an indicator of method precision.	Over the course of the project, approximately 1 per 20 samples.

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 8. Laboratory QC Requirements

Parameter	**Method Blank**	**Lab Duplicate RPD**	**Negative Control**	**CS % Recovery**	**Spike Blank**	**Matrix Spike**
Chl-*a*	<MDL	25%	NA	90 - 110 %	NA	NA
Pheo-*a*	<MDL	50%	NA	NA	NA	NA
Microcystins	<MDL	Performance based[b]	<0.1 ppb	NA	Performance based	Performance based
Quantitative[a]. Phytoplankton	NA	NA	NA	NA	NA	NA

Notes:
CS- Check Standard (positive control equivalent to Laboratory Control Sample)
MDL – Method Detection Limit
NA – Not Applicable
RPD – Relative Percent Difference
a. QC will be defined when method validation has been completed.
b. This control limit applies to matrix spike / matrix spike duplicate RPD.

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Spreadsheet A.
2007 Toxic Cyanobacteria Sampling Schedule

Major Lakes and Toxic Cyanobacteria Phytoplankton Sampling Schedule

IC = Integrated composite sample ("the tube")
S = Discrete surface sample
c = chlorophyll

H = Collect quantitative phytoplankton samples and hold pending microcystin values.
Q = Quantitative phytoplankton analysis.
M = microcystin

NOTE: ALL sites have chlorophyll samples

			0826	0852 [a]	0831	0807	4903	0834	0817	0832	625	0611	0612	0614	A522
Sampling Method =>			IC	IC	IC	S	S	S	S	S	S	IC	IC	S	IC
JAN	1 sample		c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc
FEB	1 sample		c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc
MAR	2nd week	12-Mar	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	26-Mar	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
APR	2nd week	9-Apr	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	23-Apr	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	5th week														
MAY	2nd week	14-May	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	29-May	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
JUN	2nd week	11-Jun	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	25-Jun	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
JUL	2nd week	9-Jul	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	23-Jul	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	5th week														
AUG	2nd week	13-Aug	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	27-Aug	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
SEP	2nd week	11-Sep	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	25-Sep	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
OCT	2nd week	8-Oct	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	4th week	22-Oct	HcM	QcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	HcM	QcM	HcM	QcM
	5th week														
NOV	1 sample		c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc
DEC	1 sample		c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc

a. One field replicate sample collected at 0852 for microcystin, chlorophyll, **and quantitative phytoplankton analysis** every other sampling event.

			806SB	826SB	4903SB	834SB	83930SB	828SB	852SB	818SB	602SB	615SB
Sampling Method =>			S	S	S	S	S	S	S	S	S	S
JAN	none											
FEB	none											
MAR	none											
	none											
APR	none											
	none											
	none											
MAY	1st week											
	3rd week	21-May	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
JUN	1st week	4-Jun	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	18-Jun	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
JUL	1st week	2-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	16-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	5th week	30-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
AUG	1st week	6-Aug	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	20-Aug	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
SEP	1st week	3-Sep	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	17-Sep	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
OCT	none											
	none											
	none											
NOV	none											
DEC	none											

a One field replicate sample collected at 0806SB for microcystin analysis every other sampling event.