# ADDENDUM TO PHASE IV SAMPLING AND ANALYSIS PLAN FOR MAJOR LAKES TOXIC CYANOBACTERIA

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**NAME OF PROJECT**: Sampling and Analysis Plan for Major Lakes Toxic Cyanobacteria.

**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 IV Toxic Cyanobacteria Sampling and Analysis Plan (SAP) study design are noted below. These modifications were made in an effort to streamline costs to the program by focusing efforts at areas of greatest public health risks, the swimming beaches. Measuring cyanotoxins at the pelagic stations of lakes Union, Washington, and Sammamish have been eliminated due to budget cuts and program revisions. Table 1 lists major changes to the Toxic Cyanobacteria program since 2003 as documented in sequential SAPs.

**Table 1. Major Changes Made To The Toxic Cyanobacteria Program Since 2003.**

| | |
|---|---|
| **2005 Phase II** | - Ten swimming beach sites were added mid-May – mid-Sept<br>- Seven nearshore routine Major Lake sites were added that correspond to selected swimming beach locations.<br>- Combined cell lysing methods. Each sample is frozen, thawed, and then sonicated. |
| **2006 Phase II Addendum** | - The number of quantitative phytoplankton samples being analyzed routinely was reduced to stations 0852, 0612, and A522. Nearshore samples and swimming beach samples sent for analysis depending upon microcystin results. |
| **2007 Phase III** | - Swim beach station A734WSB on Green Lake was added.<br>- Phytoplankton sample bottle size was changed from 1 x 60ml and 1 x 500ml, to 1 x 60ml and 1 x 250ml.<br>- HPLC verification eliminated |
| **2008 Phase IV** | - Anatoxin-a method development added.<br>- Stations modified to include all swimming beaches monitored as part of the Swimming Beach program. This does not include beaches contracted by other jurisdictions.<br>- Nearshore Major Lake stations dropped.<br>- KCEL completed their quantitative phytoplankton method development. |
| **2009 Phase IV Addendum** | - PPIA was dropped. ELISA the only method used to detect microcystins.<br>- Quantitative phytoplankton samples contracted to WATER Environmental.<br>- Anatoxin-a routinely analyzed on samples collected every other sampling event.<br>- Pelagic Major Lake Stations dropped from the cyanotoxin study. |

## 1.1 Project Objectives

The objectives of the Phase IV Cyanobacteria Toxicity Program as of this addendum are to:

- Evaluate the presence of microcystins, a hepatoxin, and anatoxin-a, a neurotoxin, to protect human health
- Estimate concentrations and geographic extent of the toxicity, should it be present
- Establish relationships between microcystin and cyanobacteria species/abundance.

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 human health.

## 1.2 Approach

The Phase IV survey was primarily designed to evaluate the presence/absence of the cyanobacterial toxins microcystins and anatoxin-a, and secondarily to estimate concentrations and geographic extent of the toxicity, should it be present. This addendum eliminates routine cyanotoxin monitoring from the pelagic stations in the three major lakes. Sampling and analysis will continue at the 17 beaches involved in the Swimming Beach Monitoring Program. The monitoring efforts described in this Addendum to the Phase IV SAP began in May 2009.

Sample collection will utilize the combined efforts of the Swimming Beach Monitoring Program. Table 2 lists, and Figure 1 illustrates, the 17 Swimming Beach sampling sites included in this cyanotoxicity monitoring effort. Table A (attached) lists all Major Lake and Swimming Beach sampling sites, weeks sampled, and illustrates how cyanotoxin analysis is coordinated with phytoplankton and zooplankton sample collection and analysis. Swimming Beach Monitoring occurs weekly from mid-May through mid-September. Because high cyanotoxin concentrations have often been detected in Washington State in early autumn, additional sampling at the swimming beach sites will take place in October.

Microcystin analysis will be done weekly on every sample. Anatoxin- *a* analysis will be done on samples every other week. Quantitative phytoplankton samples will be collected at each site listed in Table 2 and held for analysis pending measured concentrations of cyanotoxins.

**Table 2. Cyanotoxin Stations and Sampling Parameters at Each Location.**

| Station | Locator | Parameter[1] | Annual # samples/parameter |
|---|---|---|---|
| 130th Place | 0805ASB | M/H/A | 25/25/13 |
| Juanita | 0806SB | M/H/A | 25/25/13 |
| Seward Pk. | 0813SB | M/H/A | 25/25/13 |
| Matthews | 0818SB | M/H/A | 25/25/13 |
| Mt Baker | 0820SB | M/H/A | 25/25/13 |
| Magnuson | 0826SB | M/H/A | 25/25/13 |
| Magnuson Off Leash | 0826OLA | M/H/A | 25/25/13 |
| G. Coulon | 0828SB | M/H/A | 25/25/13 |
| Meydenbauer | 0834SB | M/H/A | 25/25/13 |
| Newcastle | 83930SB | M/H/A | 25/25/13 |
| Madison Park | 0852SB | M/H/A | 25/25/13 |
| Madrona | SD007SB | M/H/A | 25/25/13 |
| L. Burbank | SD017SB | M/H/A | 25/25/13 |
| Green Lk | A734WSB | M/H/A | 25/25/13 |
| Pritchard | 4903SB | M/H/A | 25/25/13 |
| Idylwood | 0602SB | M/H/A | 25/25/13 |
| Lk Samm Park | 0615SB | M/H/A | 25/25/13 |

*PARAMETER CODES:
M = microcystin analysis
H = collect quantitative phytoplankton samples and hold for analysis pending cyanotoxin values.
A = anatoxin-a analysis

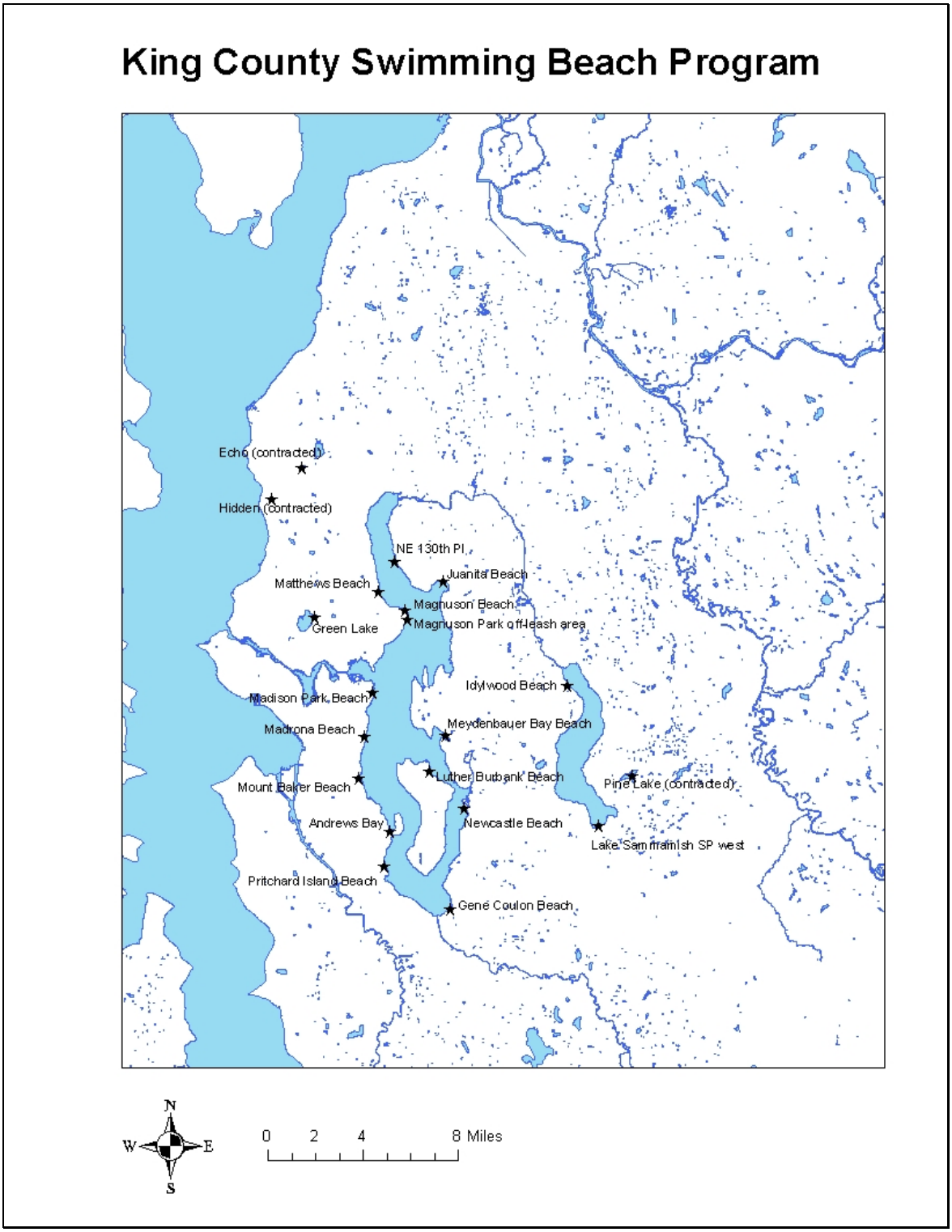


**Figure 1. Sampling Locations for the King County Swimming Beach Monitoring Program.**

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

1. Swimming Beach Monitoring. The major component of this sampling scenario will be conducted by the laboratory's Environmental Services Section (ESS) staff as part of the Swimming Beaches Monitoring Program. The seventeen swimming beaches that are part of the seasonal monitoring for fecal coliform bacteria are included in this Addendum to the Phase IV of the Toxic Cyanobacteria Study (Table 2).

   Microcystin will be measured by ELISA using the freezing and sonication extraction method (described in the Phase II SAP). In addition, samples will be collected for anatoxin-a analysis every other week. KCEL has adapted a highly sensitive method for anatoxin-a that uses fluorimetric derivatization with HPLC.

   Sufficient sample volume will be collected for microcystin testing, quantitative phytoplankton identification and enumeration, and anatoxin-a when indicated. Quantitative phytoplankton identification and enumeration samples collected from swimming beaches will be archived and analyzed if determined necessary by high cyanotoxin concentrations. If toxins are present, samples will be scanned by DNRP staff (Sally Abella) and potentially sent for quantitative phytoplankton identification and enumeration. Quantitative phytoplankton identification and enumeration will 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. Up to 10 samples may be collected per bloom event, followed by Project Manager evaluation, and subsequent decisions regarding appropriate next steps.

2. Bloom Sampling. Focused sampling efforts will be made to collect blooms or accumulations of cyanobacteria if they are present within the visual distance of routine lakes sampling sites or as part of the Small Lakes Monitoring Program. 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 and/or Small Lakes Programs. 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, as well as phytoplankton quantitative enumeration and identification. Microcystin will be measured by ELISA and Anatoxin-a will be measured by HPLC. If toxins are present, quantitative phytoplankton identification and enumeration may be determined.

**NOTE** that quantitative phytoplankton enumeration and identification will be performed for samples collected by integrated composite at A522, 0852, and 0612 as part of the Major Lake (see Major Lakes Monitoring SAP, forthcoming in 2009). Zooplankton samples will be collected at stations A522 and 0612. WATER Environmental Services will conduct the analysis of both quantitative phytoplankton and zooplankton. Data from these plankton analyses will be stored in a databases being established by WLRD's Science Section.

### 1.3 Timeline

As noted, initial routine sampling and analysis of microcystins by ELISA was implemented in spring, 2002. This was followed by several modifications of the SAP as noted in the latest Phase IV SAP produced in June 2008. Modifications to the Phase IV SAP, as outlined in this addendum, will be implemented in March 2009.

### 1.4 Sampling Procedures

The following sampling procedures are based on methods of Carmichael (2001), Chorus (2001), Johnston and Jacoby (2002).

**Table 3. Sample Container & Preservation Requirements**

| Parameter | Matrix | Container | Preservation | Hold time |
|---|---|---|---|---|
| Phytoplankton | Liquid | •1x 60- ml Plastic Amber Wide Mouth (AWM) | •Lugol's solution pre-preserved<br>•Store at room temperature in the dark | •365 days |
| Microcystins ELISA<br>(MLR-ELISA) | Liquid | 250- ml Glass, AWM | 4°C | 24 to 48 hours then freeze |
| Anatoxin-a HPLC | Liquid | 2x 1– L Plastic Amber bottle (2 per station) | 4°C | 1 day for filtration<br>28 days for analysis |

Notes:
AWM – Amber wide mouth bottle
HPLC – High performance liquid chromatography

#### 1.4.1 Water sample collection and storage procedure to test for toxins:

Swimming Beach cyanotoxin and phytoplankton samples will be collected by the following method.

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 1 ½ feet below the surface, leaving some headspace to allow for freezing.

Other sampling protocol notes:

- Label the bottles if not pre-labeled.
- Place the sample bottles in a cooler with ice packs (no preservative required).
- For Microcystins, sub-samples 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.

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

Samples for quantitative identification and enumeration will be collected and preserved at the Swimming Beach sites in the event that high microcystin and/or anatoxin-a concentrations warrant further investigation. A 60 mL aliquot will be collected and placed in properly labeled opaque bottles (typically 60 mL plastic amber wide mouth) and preserved with a sufficient amount of concentrated Lugol's solution to turn the sample light red; typically 0.4 ml Lugol's is the preferred amount for 60 mL bottle. Care should be taken that samples are covered tightly and stored in the dark until analyzed.

In the event that algal blooms are sampled (as per section 1.2), samples will be collected and preserved as described above.

## 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 extracellular microcystins at concentrations at or above 0.05 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. The KCEL has developed Standard Operating Procedures (SOPs) for the measurement of microcystins using ELISA (SOP 440v2) and microcystins and nodularins using PPIA (SOP 443v1) in water.

As of this addendum, KCEL will discontinue PPIA analysis at the swimming beaches.

## 2.1 Microcystin 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*. A recent study also identified microcystin-LR production by *Gloeotrichia echinulata* (Carey et al. 2007). These toxins contain two variable L-amino acids, three D-amino acids and two unusual amino acids. Microcystins are the most thoroughly investigated cyanobacterial toxins (Falconer 2005). At least 71 structural variants have been identified, and microcystin-LR is the variant most commonly found in cyanobacteria (Huisman et al. 2005, Botana 2007). 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. KCEL currently uses an Envirologix ELISA plate kit. 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.

### 2.1.1 Sample Preparation for Microcystin 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, 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 *Extracellular Microcystins.* Since all samples collected for this study will be analyzed following lysing, results will be reported as *Total Microcystins*. Note ELISA measures only free microcystin, not the amount chemically bound to the cell or molecular components such as protein phosphatase enzymes.

Based on previous cyanobacteria toxin work done, laboratory staff recommended combining extraction methods. Therefore, each sample will be prepared for analysis by 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 nominal molecular weight limit (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 centrifugation, may be most efficient (see attachment for further discussion). The method detection limit (MDL) is 0.05 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) 440v2 and 443v1, 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 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. Anatoxin-a Toxin Structure and Cross-Reactivity Analysis Summary

The neurotoxin anatoxin-a is an alkaloid with high toxicity ($LD_{50}$ i.p. mouse 200 µg/kg). It acts as a post-synaptic, depolarizing, neuromuscular blocking agent. There is evidence that anatoxin-a can be produced by some species of *Anabaena*, *Aphanizomenon, Cylindrospermum* and *Oscillatoria*.

The determination of anatoxin-a in natural waters has been challenging due to its typically low concentration. The highly sensitive method adapted at KCEL uses fluorimetric derivatization with HPLC. Anatoxin-a is converted into a fluorescent derivative using 4-fluoro-7-nitro-2,1,3-benzoxadiazole and the fluorescent compound is then separated and detected by HPLC. Further increase in sensitivity is accomplished by concentrating the algal cells on a filter. This method will thus only measure intracellular toxin. Since anatoxin-a is easily degraded once outside the cell, we can assume that the filtration step will retain most or all of the active toxin present in the sample.

### 2.1.3 Sample Preparation for Anatoxin-a Toxin Assay

Anatoxin-a degrades readily, especially in sunlight and at high pH. At KCEL the bottles are immediately transferred to a 4 °C chamber. The samples are filtered onto glass fiber filters (45 mm Whatman 934-AH) within 24 hours of arrival and the filters stored in cryovials at -20 °C (2 filters per station). The filtrate is discarded.

KCEL developed a SOP for the measurement of anatoxin-a in water samples using isocratic HPLC with fluorescence detection in 2008 (SOP 457). The reference method is James et al. (1998).

Extraction: Anatoxin-a is extracted from cells by treating the filter with 100% methanol (freezing and sonication). The extract may or may not be dried prior to derivatization. Alternatively, a whole water sample is extracted, cleaned and concentrated using a weak cation exchange solid phase extraction column. The resulting extract is evaporated under nitrogen and stored at -20 °C.

Derivatization: Dried samples or standards are derivatized with 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) following the method of James et al. (1998). The reaction is terminated at 60 minutes, when samples are centrifuged or filtered and transferred to autosampler vials.

Chromatography: HPLC analysis is performed with an Agilent 1200 series system using a Zorbax $C_{18}$ column, 55% acetonitrile-water as the mobile phase and fluorimetric detection at 470 nm (excitation) and 530 nm (emission). Run time is 45 min per injection. The system can be programmed to run a complete batch overnight (e.g., blank, 5 standards, 2 spikes, 5-8 samples).

Anatoxin-a analytical standards are purchased commercially, diluted and run along with samples to create a 5-point calibration curve (0.05 – 0.50 µg/L). Methyl pipecolinate (1.25 mg/L) is added to all samples and standards and used as an internal or reference standard. Dihydroanatoxin and epoxyanatoxin (non-toxic anatoxin-a degradation products) can be synthesized in house and added to the standards. Natural samples spiked with anatoxin-a will also be run routinely.

The MDL for anatoxin-a in ambient water will typically be 0.0029 ug/L when 1 L of sample is analyzed. Strong matrix effects could, however, significantly affect the level of detection for certain samples. Washington State set a provisional guidance value of 1 ug/L anatoxin-a (Washington Department of Health, 2008).

## 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 |
|---|---|---|---|
| Microcystins by ELISA | KCEL SOP 440v2 | 0.05 µg/L | 0.05 µg/L |
| Anatoxin-a by HPLC | KCEL SOP 457v0 | 0.0029 ug/L | 0.0029 ug/L |

# 3 Data Quality Objectives

The procedures and practices described in this study specific SAP Addendum are designed to generate data of sufficient quality to support decision making as discussed above and in the Major Lakes Monitoring Program SAP; King County, (forthcoming in 2009). 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 6.0, Quality Control Procedures, addresses many of the procedures necessary to obtain data that meet these data quality objectives.

### 3.1.1 Laboratory Precision

Laboratory precision will be assessed using laboratory duplicate 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.

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

### 3.1.2 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 SAP.

### 3.1.3 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.

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.

### 3.1.4 Representativeness

This survey is primarily designed to evaluate the presence/absence of cyanobacterial toxicity. 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, forthcoming in 2009.

### 3.1.5 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 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.

### 3.1.6 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 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.

# 4 Data Reduction, Review, and Reporting

Data reduction, review and reporting will be performed under the KCEL's standard operating procedures. Laboratory data will be provided to data recipients within 5 days of sample receipt or within 30 days of the decision to analyze archived samples. Data reports will include sufficient information to conduct the data assessment. Data will be reported in the standard laboratory-reporting format. This includes an analytical result, MDL and RDL, if available.

Protocols have been worked out 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 as illustrated in the attached decision making flowchart (see Attachment C). 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 may include the following:

- KCEL LIMSView Reports consisting of spreadsheets of analytical and field parameters

# 5 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 |
|---|---|---|---|
| Colin Elliott<br>(206) 684-2343 | Laboratory Project Manager | Environmental Laboratory | Coordination of analytical activities, lab QA/QC and data reporting. |
| Judy Ochs<br>(206) 684-2347 | Environmental Scientist | Environmental Laboratory | Coordination of sampling activities, field QA/QC, and field analysis for the Swimming Beach Program |
| Debra Bouchard<br>(206) 263-6343 | Water Quality Planner | Water & Land Resources | Project manager for the Toxic Cyanobacteria Study, coordination between various groups at the lab |
| Fran Sweeney<br>(206) 684-2358 | AquaTox Lab<br>Supervisor | Environmental Laboratory | Coordination with REHAB and DOE Algae Control Projects |
| Gary Yoshida<br>(206) 684-2312 | Aquatic Toxicologist | Environmental Laboratory | Coordination of microcystin analysis; |
| Jim Buckley<br>(206) 684-2314 | Aquatic Toxicologist | Environmental Laboratory | Coordination of anatoxin-a |

# 6 Quality Control Procedures

## 6.1 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 project, 1 per sampling event. |

### 6.1.1 QC Practices for Field Measurements

Sampling for this Toxic Cyanobacteria Study is conducted concurrently with the Swimming Beach Monitoring Program. Therefore QA practices are covered under those SAPs/SOPs.

## 6.2 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. Additionally, the KCEL participates regularly in US EPA inter-laboratory performance evaluation studies.

### 6.2.1 Frequency of quality control samples

All samples will be analyzed at the KCEL. 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 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 analytical process and used as an indicator of contamination. | 1 per sample batch. Maximum sample batch size equals 20 samples or one 96-well ELISA plate. |
| Laboratory Control | Solution of known analyte concentration, processed through the entire analytical | 1 per sample batch, as available. Maximum |

| | | |
|---|---|---|
| Sample (LCS) | procedure and used as an indicator of method accuracy and precision. | sample batch size equals 20 samples or one 96-well ELISA plate. |
| 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 or one 96-well ELISA plate. . |
| Negative Control (NC) | A solution obtained from the ELISA kit manufacturer with a confirmed microcystin concentration of zero. | 1 per ELISA instrument run. |
| Positive Control | A standardized spike of the primary ELISA toxin, typically mid-standard curve. | 1 per ELISA instrument run. |
| 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 or one 96-well ELISA plate.<br><br>Note, MS/MSD may be dropped for bloom samples due to interferences from high concentrations of toxins and necessary dilutions. |
| Spike Blank (SB) | 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. | 1 per sample batch. Maximum sample batch size equals 20 samples. or one 96-well ELISA plate. |
| 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 week. Note that RPDs may be of limited usefulness if <MDL. |

KCEL laboratory QC samples for 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 | Positive Control (% Recovery) | Negative Control | Spike Blank | Matrix Spike |
|---|---|---|---|---|---|
| Microcystin | <MDL | Performance Based, current limits approx:<br><br>95-125 | <0. 05 ug/L | Performance Based, current limits approx:<br><br>80-150 | Performance Based, current limits approx:<br><br>50-150 |
| Anatoxin a | <MDL | NA | NA | 80-120 | 80-120 |

Performance based QC requirements rely on the development of statistically derived control limits (ex. mean response ± 3SD) in absence of EPA or DOE mandated control limits for an analytical method.

## 6.3 Corrective Action

KCEL 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 SAP, the lab will work with the data user to develop and implement corrective action.

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# 8 Attachment A. SAMPLING SCHEDULES FOR MAJOR LAKES PLANKTON AND SWIMMING BEACH CYANOBACTERIA PROGRAMS

**Major Lakes Phytoplankton and Zooplankton Sampling Schedule**

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

Z = Zooplankton collected from tows as indicated in footnotes. Sent to WATER Environmental for analysis.
Q = Quantitative phytoplankton analysis from Integrated composite sample. Sent to WATER Environmental for analysis.

**NOTE:** **ALL sites have chlorophyll samples**

| | | | 0826 | 0852 [a] | 0831 | 0804 | 0512 | 0540 | A522 [b] | 0611 | 0612 [c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling Method/depths for chla a => | | | IC | IC/1, 5, 10, 15, 20, 25 | IC | S | S | S | IC | IC | IC/1, 5, 10, 15, 20 |
| JAN | 1 sample | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| FEB | 1 sample | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| MAR | 2nd week | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 4th week | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| APR | 2nd week | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 4th week | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| MAY | 1st week | 5-May | | | | | | | | | |
| | 2nd week | 11-12 May | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 3rd week | 19-May | | | | | | | | | |
| | 4th week | 25-26 May | c | Qc | c | c | c | c | ZQc | c | ZQc |
| JUN | 1st week | 2-Jun | | | | | | | | | |
| | 2nd week | 8-9 Jun | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 3rd week | 16-Jun | | | | | | | | | |
| | 4th week | 22-23 Jun | c | Qc | c | c | c | c | ZQc | c | ZQc |
| JUL | 1st week | 30-Jun | | | | | | | | | |
| | 2nd week | 7-Jul | | | | | | | | | |
| | 3rd week | 13-14 Jul | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 4th week | 21-Jul | | | | | | | | | |
| | 5th week | 27-28 Jul | c | Qc | c | c | c | c | ZQc | c | ZQc |
| AUG | 1st week | 4-Aug | | | | | | | | | |
| | 2nd week | 10-11 Aug | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 3rd week | 18-Aug | | | | | | | | | |
| | 4th week | 24-25 Aug | c | Qc | c | c | c | c | ZQc | c | ZQc |
| SEP | 1st week | 31-Aug | | | | | | | | | |
| | 2nd week | 8-Sep | | | | | | | | | |
| | 3rd week | 14-15 Sept | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 4th week | 22-Sep | | | | | | | | | |
| | 5th week | 28-29 Sept | c | Qc | c | c | c | c | ZQc | c | ZQc |
| OCT | 1st week | 6-Oct | | | | | | | | | |
| | 2nd week | 12-13 Oct | c | Qc | c | c | c | c | ZQc | c | ZQc |
| | 3rd week | 20-Oct | | | | | | | | | |
| | 4th week | 26-27 Oct | c | Qc | c | c | c | c | ZQc | c | ZQc |
| NOV | 1 sample | | c | Qc | c | c | c | c | ZQc | c | ZQc |
| DEC | 1 sample | | c | Qc | c | c | c | c | ZQc | c | ZQc |

a. One field replicate sample collected at 0852 for microcystin, chlorophyll, and quantitative phytoplankton analysis every other sampling event.
b. Zooplankton tow: 10 meters to 0.
c. Zooplankton tows: 25 meters to 18, 20 to 10, and 10 to 0. One qualitative tow with #20 net.

**Swimming Beach Cyanotoxin Sampling Schedule**

**** samples will be collected on alternate weeks from Major Lakes sampling**

S = Discrete surface sample
H = Collect quantitative phytoplankton samples and hold pending microcystin values.
a = anatoxin

M = microcystin

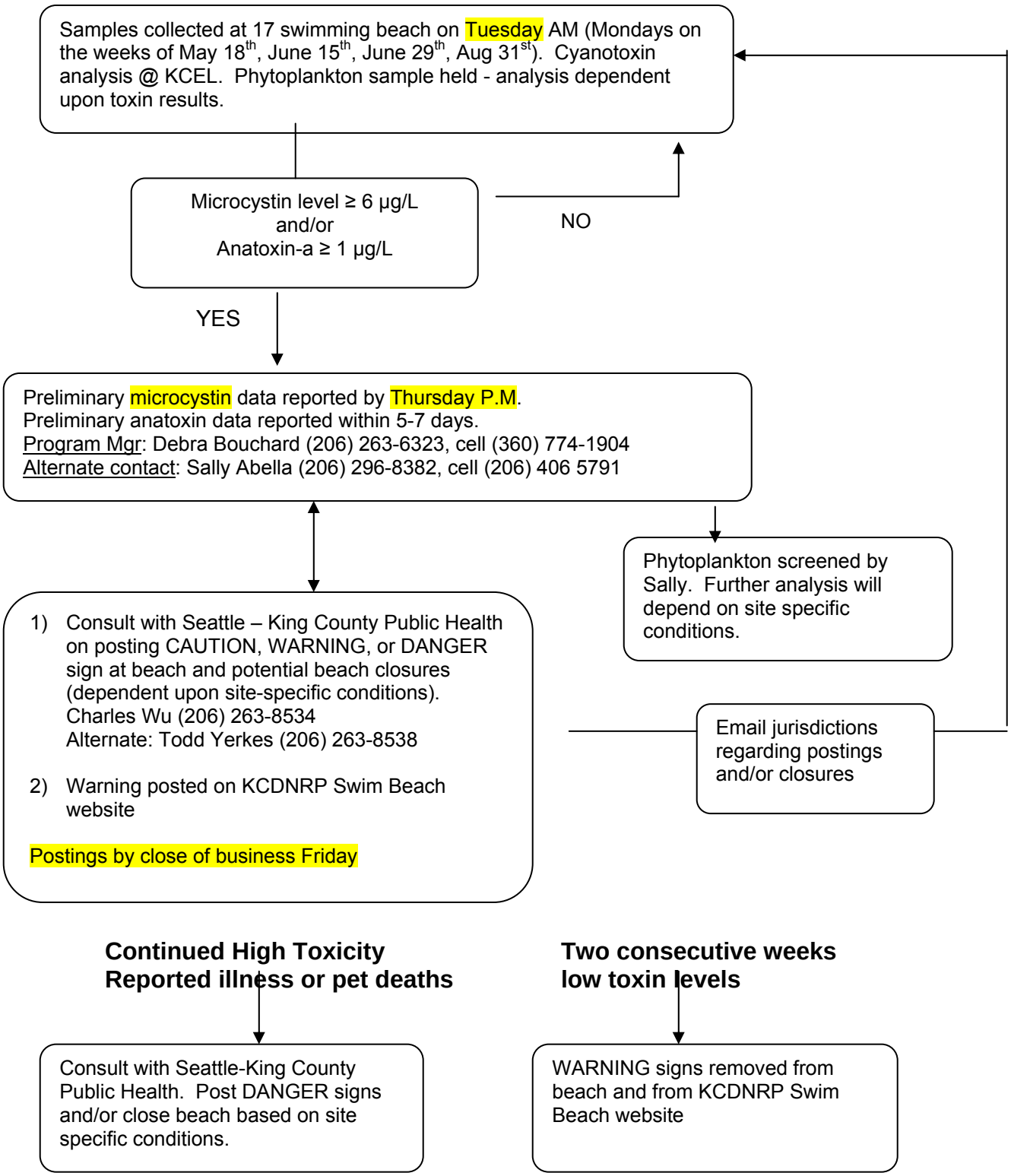
**NOTE: phytoplankton analysis only if high microcystin concentrations**

| | | | 0805ASB | 806SB* | 0813SB | 0818SB | 0820SB | 826SB | 826OLA | 828SB | 834SB | 83930SB | 852SB | A734WSB | SD007SB | SD017SB | 4903SB | 602SB | 615SB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling Method => | | | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| JAN | 1 sample | | | | | | | | | | | | | | | | | | |
| FEB | 1 sample | | | | | | | | | | | | | | | | | | |
| MAR | 2nd week | | | | | | | | | | | | | | | | | | |
| | 4th week | | | | | | | | | | | | | | | | | | |
| APR | 2nd week | | | | | | | | | | | | | | | | | | |
| | 4th week | | | | | | | | | | | | | | | | | | |
| MAY | 1st week | | | | | | | | | | | | | | | | | | |
| | 2nd week | 12-May | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 3rd week | 19-May | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 4th week | 26-May | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| JUN | 1st week | 2-Jun | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 2nd week | 9-Jun | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 3rd week | 16-Jun | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 4th week | 23-Jun | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| JUL | 1st week | 30-Jun | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 2nd week | 7-Jul | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 3rd week | 14-Jul | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 4th week | 21-Jul | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 5th week | 28-Jul | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| AUG | 1st week | 4-Aug | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 2nd week | 11-Aug | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 3rd week | 18-Aug | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 4th week | 25-Aug | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| SEP | 1st week | 1-Sep | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 2nd week | 8-Sep | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 3rd week | 15-Sep | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 4th week | 22-Sep | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 5th week | 29-Sep | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| OCT ** | 1st week | 6-Oct | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 2nd week | 13-Oct | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| | 3rd week | 20-Oct | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH | MH |
| | 4th week | 27-Oct | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa | MHa |
| NOV | 1 sample | | | | | | | | | | | | | | | | | | |
| DEC | 1 sample | | | | | | | | | | | | | | | | | | |

* One field replicate sample collected at 0806SB for microcystin analysis every other sampling event.
** Samples collected beyond swim beach program

**Major Lakes and Swimming Beach Programs**
**Phytoplankton, Zooplankton, and Cyanotoxin Analysis**

**Total Samples Collected per Sampling Week**

| | | # Phytos ANALYZED | # Zoops ANALYZED | # Microcystins | # Anatoxin |
|---|---|---|---|---|---|
| | | per sampling week | per sampling week | per sampling week | per sampling week |
| JAN | 1 sample | 3 | 4 | | |
| FEB | 1 sample | 3 | 4 | | |
| MAR | 2nd week | 3 | 4 | | |
| | 4th week | 3 | 4 | | |
| APR | 2nd week | 3 | 4 | | |
| | 4th week | 3 | 4 | | |
| MAY | 1st week | | | | |
| | 2nd week | 3 | 4 | 17 | 17 |
| | 3rd week | | | 17 | |
| | 4th week | 3 | 4 | 17 | 17 |
| JUN | 1st week | | | 17 | |
| | 2nd week | 3 | 4 | 17 | 17 |
| | 3rd week | | | 17 | |
| | 4th week | 3 | 4 | 17 | 17 |
| | 5th week | | | 17 | |
| JUL | 1st week | | | 17 | 17 |
| | 2nd week | 3 | 4 | 17 | |
| | 3rd week | | | 17 | 17 |
| | 4th week | 3 | 4 | 17 | |
| AUG | 1st week | | | 17 | 17 |
| | 2nd week | 3 | 4 | 17 | |
| | 3rd week | | | 17 | 17 |
| | 4th week | 3 | 4 | 17 | |
| SEP | 1st week | | | 17 | 17 |
| | 2nd week | 3 | 4 | 17 | |
| | 3rd week | | | 17 | 17 |
| | 4th week | 3 | 4 | 17 | |
| | 5th week | | | 17 | 17 |
| OCT | 1st week | | | 17 | |
| | 2nd week | 3 | 4 | 17 | 17 |
| | 3rd week | | | 17 | |
| | 4th week | 3 | 4 | 17 | 17 |
| NOV | 1 sample | 3 | 4 | | |
| DEC | 1 sample | 3 | 4 | | |
| Annual Total | | 60 | 80 | 425 | 221 |

# 9 Attachment B. CYANOTOXIN FLOWCHART FOR THE KING COUNTY SWIMMING BEACH PROGRAM