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Quality Assurance Project Plan

Blue-Green Algae Toxins in Fish and Sediment from Washington Lakes: Microcystins and Saxitoxin

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Quality Assurance Project Plan

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September 2010

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Signatures are not available on the Internet version.
PDS-TSU: Program Development Services Section, Technical Services Unit.
EAP: Environmental Assessment Program.
EIM: Environmental Information Management system.

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Abstract

Each study conducted by the Washington State Department of Ecology must have an approved Quality Assurance (QA) Project Plan. The plan describes the study objectives and procedures to be followed to achieve them. After completing the study, Ecology will post the final report of the study to the Internet.

This QA Project Plan describes a study to analyze microcystins and saxitoxin in fish tissue samples from selected Western Washington lakes that experience blue-green algae (cyanobacteria) blooms during the summer and fall of 2010. Sediments from several of the lakes will also be analyzed for microcystins. These blue-green toxins can affect the liver (microcystins) or nervous system (saxitoxin) of animals, including humans. The impetus for this work is their detection in fish collected from six local lakes in 2008.

Background

In 2008, the Washington State Department of Ecology (Ecology) tested for the presence of microcystins and anatoxin-a in fish from six Western Washington lakes that were experiencing blue-green algae (cyanobacteria) blooms. These compounds are highly toxic to animals, including humans, and are an emerging public health issue. Microcystins primarily affect the liver. Anatoxin-a is a neurotoxin. Until recently, the primary exposure pathways of concern have been through drinking water and recreational exposure. Information on the biology and toxicity of blue-greens can be found on the Washington State Department of Health (WDOH) cyanobacteria website (www.doh.wa.gov/ehp/algae/whatarecyanobacteria.htm) and Ecology's Freshwater Algae Control Program website (www.ecy.wa.gov/programs/wq/plants/algae/index.html).

Potentially significant concentrations of microcystins were found in fish from most of the lakes surveyed in 2008 (Johnson, 2010). The accuracy of the microcystin analysis, however – which was by an enzyme-linked immunosorbent assay (ELISA) – was difficult to assess. Anatoxin – analyzed by high pressure liquid chromatography (HPLC) – was not detected. It is apparently too unstable to accumulate or is simply not taken up by fish. A third blue-green toxin, saxitoxin, was tentatively identified by ELISA in fish from the one lake it was tested for. Saxitoxin is a neurotoxin primarily associated with paralytic shellfish poisoning (PSP or "red tide").

The report on the 2008 study recommended several follow-up studies focused on microcystins and saxitoxin. This work will be undertaken in two phases. The present Quality Assurance (QA) Project Plan is for a Phase I study to obtain better data on microcystin levels in fish, to analyze microcystins in lake sediments, and to screen selected fish samples for saxitoxin. The study will employ both liquid chromatography/mass spectrometry (LC/MS) and the more rapid and less costly ELISA method, in part to evaluate ELISA's accuracy for use on microcystins in Phase II.

Phase II will analyze microcystins in a series of water and fish samples collected from several lakes at regularly timed intervals prior to, during, and following blue-green blooms to better determine the extent and duration of elevated concentrations. This work is tentatively planned to begin in the spring of 2011 to give enough lead time to establish pre-bloom conditions and to benefit from experience gained with ELISA through the present study. A separate QA Project Plan will be prepared for that effort.

Project Description

For Phase I, microcystins will be analyzed in fillets from fish collected in association with blue-green algae blooms in Western Washington lakes during the summer and fall of 2010. The lakes and species sampled will depend on where significant blooms occur and their severity. The trigger for fish sampling will be high levels of microcystins in algae samples collected through Ecology's Freshwater Algae Control Program www.ecy.wa.gov/programs/wq/plants/algae/monitoring/index.html.

Ten blooms are budgeted for, assuming two tissue samples per bloom, on average. Bottom sediments and beach material from five lakes with a history of severe blooms will also be analyzed for microcystins, three samples per lake. Five fish samples from a known saxitoxin producing lake will be screened for saxitoxin.

Microcystins will be analyzed by both LC/MS and ELISA methods. ELISA will be used for saxitoxin.

Objectives of the Phase I study are to:

1. Obtain accurate, verifiable data on microcystins in edible fish tissue.
2. Evaluate the accuracy of the ELISA method for fish tissue.
3. Assess the persistence of microcystins in lake sediments and on beaches.
4. Obtain estimates of saxitoxin levels in fish from a lake subject to saxitoxin blooms.
5. Aid WDOH in determining if microcystins and saxitoxin represent a human health concern for fish consumers or through recreational contact with lake sediments or beaches.

The Ecology Environmental Assessment (EA) Program will lead the study and prepare the project report. Fish and sediment samples will be collected by the EA Program, with assistance on fish collection from the Washington Department of Fish and Wildlife (WDFW). The samples will be analyzed by the Water Pollution Control Laboratory of the California Department of Fish & Game (CDFG; LC/MS analyses) and King County Environmental Laboratory (KCEL; ELISA analyses). The data will be provided to the WDOH Office of Environmental Health, Safety, and Toxicology for their use in assessing human health concerns.

This study was requested by the Ecology Water Quality Program, Program Development Services Section, Technical Services Unit (PDS-TSU). The QA Project Plan follows the Ecology guidance in Lombard and Kirchmer (2004).

Organization and Schedule

The following people are involved in this project.

Table 1. Organization of Project Staff and Responsibilities.

Staff	Title	Responsibilities
Kathy Hamel PDS-TSU Water Quality Program, Ecology (360) 407-6562	Client	Clarifies scopes of the project. Provides internal review of the QAPP and approves the final QAPP. Reviews and approves the project report.
Joan Hardy Environmental Health Assessments, Washington State Department of Health (360) 236-3173	Toxicologist	Serves as Department of Health contact for receiving project data.
Art Johnson Toxics Studies Unit SCS, EAP, Ecology (360) 407-6766	Principal Investigator	Writes the QAPP. Coordinates fish collections and chemical analyses. Conducts QA review of data and analyzes and interprets data. Writes the project report.
Michael Friese Toxics Studies Unit SCS, EAP, Ecology (360) 407-6737	Environmentalist, EIM Data Engineer	Collects fish and assists with other field work.
Randy Coots Toxics Studies Unit SCS, EAP, Ecology (360) 407-6690	Environmentalist	Collects fish and assists with other field work.
Dale Norton Toxics Studies Unit SCS, EAP, Ecology (360) 407-6765	Unit Supervisor of Principal Investigator	Provides internal review of the QAPP and approves the final QAPP. Approves the budget. Reviews and approves the project report.
Will Kendra SCS, EAP, Ecology (360) 407-6698	Section Manager of Principal Investigator	Reviews the project scope and budget and tracks progress. Reviews the draft QAPP and approves the final QAPP.
Robert F. Cusimano Western Operations Section EAP, Ecology (360) 407-6596	Section Manager for Study Area	Reviews the project scope and budget and tracks progress. Reviews the draft QAPP and approves the final QAPP.
Stuart Magoon Ecology Manchester Environmental Laboratory, EAP (360) 871-8801	Director	Approves the final QAPP.
Karin Feddersen Ecology Manchester Environmental Laboratory, EAP (360) 871-8829	Chemist	Completes data verification review.

Staff	Title	Responsibilities
David Crane, California Dept. Fish & Game, Fish and Wildlife Water Pollution Control Laboratory (916) 358-2859	Laboratory Director	Contact for microcystin analysis by LC/MS
Fran Sweeney King County Environmental Laboratory (206) 684-2358	Supervisor AquaTox & Laboratory Project Management	Contact for microcystin and saxitoxin analysis by ELISA
William R. Kammin (360) 407-6964	Ecology Quality Assurance Officer	Reviews the draft QAPP and approves the final QAPP.

SCS: Statewide Coordination Section
EAP: Environmental Assessment Program.
EIM: Environmental Information Management system.
QAPP: Quality Assurance Project Plan.

Table 2. Proposed Schedule for Completing Field and Laboratory Work, Data Entry into EIM, and Reports.

Field and laboratory work	Due date	Lead staff
Field work completed	November 2010	Art Johnson
Laboratory analyses completed	January 2011	
Environmental Information System (EIM) database		
EIM user study ID	AJOH0061	
Product	Due date	Lead staff
EIM data loaded	May 2011	Michael Friese
EIM quality assurance	June 2011	To be determined
EIM complete	July 2011	Michael Friese
Final report		
Author lead	Art Johnson	
Schedule		
Draft due to supervisor	April 2011	
Draft due to client/peer reviewer	May 2011	
Final (all reviews done) due to publications coordinator (Joan)	June 2011	
Final report due on web	July 2011	

Quality Objectives

Quality objectives for this project are to obtain data of sufficient quality so that uncertainties are minimized and that accurate and representative results are obtained for the parameters of interest. These objectives will be achieved through careful attention to the sampling, measurement, and quality control (QC) procedures described in this plan.

Measurement Quality Objectives

As previously noted, the LC/MS and ELISA analyses will be conducted by CDFG and KCEL, respectively. KCEL is certified by Ecology's Laboratory Accreditation Program for analyzing microcystins and saxitoxin in water by the ELISA method. The CDFG laboratory is certified through the California Department of Public Health Environmental Laboratory Accreditation Program. California has no certification specifically for analyzing microcystins. California has a reciprocity agreement with the Ecology, recognizing the accreditation/certification/approval of the other as partial fulfillment of their requirements for accreditation.

KCEL and CDFG have experience with the analytical method they will be using, although modifications will be necessary to ELISA for the tissue and sediment matrices. The laboratories are expected to meet all QC requirements specified in the method.

Specific measurement quality objectives (MQOs) for the present study are shown in Table 3.

Table 3. Measurement Quality Objectives.

Analysis	Spike Blank (% recov.)	Duplicate Samples (RPD)	PCA (% recov.)	Surrogate Recovery (% recov.)	Matrix Spikes (% recov.)	Matrix Spike Duplicates (RPD)	Lowest Concentration of Interest (ug/Kg)
Fish Tissue							
Microcystins by LC/MS	50-150	≤25	NA	TBD	50-150	≤25	1 (wet)
Microcystins by ELISA	80-160	≤25	70-140	NA	80-160	≤25	1 (wet)
Saxitoxin by ELISA	70-130	≤25	70-130	NA	70-130	≤25	1 (wet)
Sediment							
Microcystins by LC/MS	TBD	TBD	NA	TBD	TBD	TBD	TBD

Note: Validation of ELISA control limits on tissue matrix will be conducted summer 2010 and limits, revised as necessary prior to project start date.
RPD: relative percent difference.
PCA: positive control assay, a spiked assay diluent sample.
TBD: to be determined.
NA: not applicable.

The recovery and precision objectives are what the laboratories anticipate being able to achieve on project samples. The lowest concentrations of interest are the low end of the range reported by other studies on blue-green toxins in lakes and reservoirs. CDFG has not yet established acceptance limits for analyzing microcystins in sediment due to limited experience with this matrix.

Ecology's Freshwater Algae Control Program

PDS-TSU oversees Ecology's Freshwater Algae Control Program. Hamel (2009) describes how the program works, portions of which follow.

When a lake resident, government staff, lake manager, or a health professional thinks a lake is experiencing an algal bloom, they contact Ecology. If Ecology decides that a bloom is likely occurring, staff explain how to collect and mail samples to the laboratory. Most often, however, staff from the local health jurisdiction collect and send in the sample. Because blue-green algal bloom distribution is often patchy within a lake, Ecology prefers that people collect samples from areas where algal scum collects, when possible.

Algae identification and toxin analysis is done by KCEL. Microcystins and saxitoxin are analyzed by ELISA and anatoxin-a is analyzed by HPLC with fluorescence detection. If the bloom is toxic—or if potentially toxin-producing blue-green algae are present—Ecology asks samplers to collect additional samples (generally on a weekly basis) for toxicity testing. In some lakes, sample collection can continue for months due to high levels of toxicity and prolonged blue-green blooms. Ecology also asks samplers to collect samples from lakes with toxic blooms for two weeks after the bloom subsides. As blooms die and decay, they often release toxins into the water that may persist even though the blue-green algae bloom is no longer visible.

The laboratory e-mails an Excel spreadsheet with results to Ecology, WDOH, and the local health district (if they collected the sample). If a lake resident sent in the sample, Ecology notifies the resident, and, if the bloom is toxic, notifies the appropriate local health authorities. If tests show that a bloom is toxic, county health officials will decide whether to post notifications of potential health concerns, close the lake for recreation, or wait for further testing. WDOH guidelines advise that a lake continue to be sampled and tested once a week for toxicity after toxin levels are above a certain concentration. Recreational use should be avoided until levels drop below the trigger concentration for two consecutive weeks. Local health officials will decide when to re-open the lake.

Within days of receiving the information, Ecology posts all results to a searchable, on-line, publicly-accessible database at https://fortress.wa.gov/ecy/toxicalgae/InternetDefault.aspx. Ecology also posts all toxic blue-green algae results to its freshwater algae electronic mailing list if the sample tests at or above the recreational guidelines for that toxin.

Study Design

Lake Selection

For logistical reasons, this study will focus on Western Washington lakes. PDS-TSU has identified 14 lakes in this region with a history of microcystin blooms and thus of potential interest for the study (Table 4, Figure 1). Saxitoxin was recently detected in algae samples from one of these lakes, Waughop in Tacoma (www.ecy.wa.gov/programs/wq/plants/algae/index.html), and was tentatively identified in fish samples collected from this lake in 2008 (Berry, 2009).

Table 4. Lakes of Potential Interest for Analyzing Microcystins and Saxitoxin.

Lake	County
American†	Pierce
Spanaway	
Steilacoom†	
Tanwax	
Ohop	
Waughop†	
Harts	
Anderson†	Jefferson
Leland†	
Gibbs	
Cassidy	Snohomish
Ketchum†	
Wilderness	King
Silver	Cowlitz

*saxitoxin to be analyzed in Waughop Lake only.
†sampled during Ecology's 2008 fish tissue study.

Data on microcystin levels in algae samples from three of the above lakes are plotted in Figure 2. This illustrates the kind of variability that can occur in blue-green activity from month to month and year to year. Note that the scales are different on the two Ketchum Lake graphs.

WDOH (Hardy, 2008) has established Washington's provisional recreational guidance value for microcystin at 6 ug/L (parts per billion). All of the lakes listed in Table 4 have substantially exceeded this value.

Ecology does not monitor saxitoxin on a routine basis. Saxitoxin was detected in a March 9, 2010 sample from Waughop Lake at a concentration of 0.57 ug/L. It was also detected separately on two other occasions under a grant from the Centers for Disease Control and Prevention, Harmful Algae Bloom-related Illness Surveillance System (Hamel, 2010; data not currently available). Washington does not have a recreational guidance value for saxitoxin in water.

Figure 1. Lakes of Potential Interest for Analyzing Microcystins and Saxitoxin (Waughop Lake) in 2010. *Silver Lake not shown; Cowlitz County, three miles east of Castle Rock.*

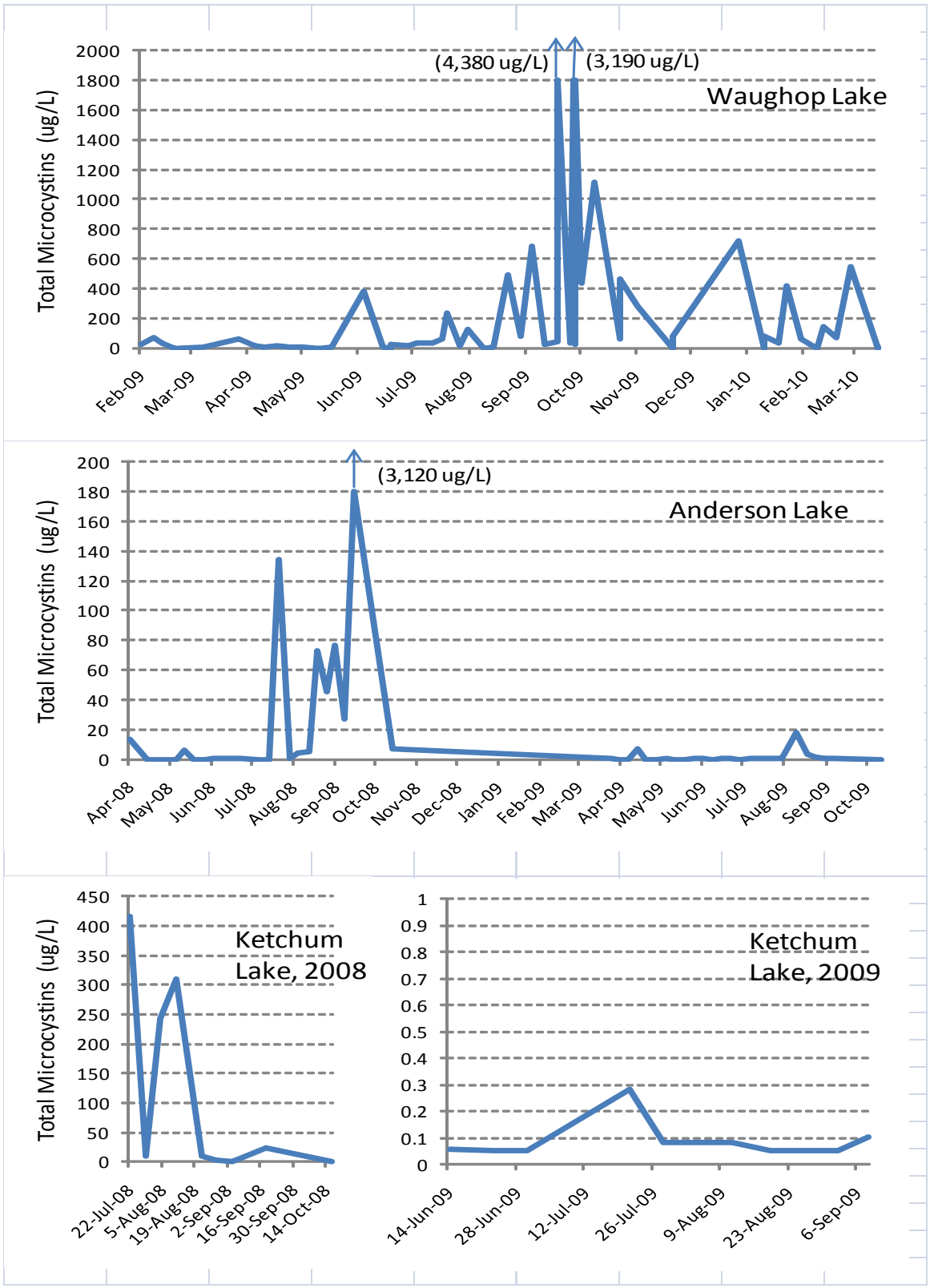

Figure 2. Total Microcystin Concentrations in Algae Samples from Three Western Washington Lakes, 2008 – 2010 (data from Ecology's Freshwater Algae Control Program).

Fish Collections

Toxin Uptake

Microcystins are released to the water, to a limited extent, by actively growing cyanobacteria. However, when cells lyse during natural bloom senescence, a significant amount of microcystins can be released (Kotak and Zurawell, 2007; Lam and Prepas 1997). Microcystins possess characteristics that should facilitate transfer between trophic levels. The compounds are relatively stable and have octanol-water partition coefficients in the range needed for bioaccumulation (White et al., 2005).

A number of studies have detected microcystins in fish tissues (e.g., Andersen et al., 1993; Magalhães et al., 2001; Zimba et al., 2001). In U.S. freshwaters, concentrations in muscle tissue have been reported in the range of 1 – 200 ug/Kg (parts per billion) wet weight (Wilson et al., 2008; Kann, 2008; Schuster et al., 2006; Carmichael, 2006a, b). Concentrations of 1 – 53 ug/Kg were found in fillets from Ecology's 2008 study (Johnson, 2010). Washington State has not established an advisory level for microcystins in fish or other types of food. WDOH is currently evaluating the 2008 data from a human health perspective.

Saxitoxin is primarily a concern in marine shellfish poisoning. No reports of saxitoxin being analyzed in freshwater fish were located. An ELISA assay of the 2008 Waughop Lake fish samples was positive for saxitoxin in the five muscle and liver samples tested (Berry, 2009). The levels, however, appeared to be low - less than 1 ug/Kg. For comparison, the regulatory action level for saxitoxin in Washington is 80 micrograms per 100 grams of shellfish. Waughop is the only known saxitoxin producing lake in Washington.

Timing, Number, and Type of Samples

It is not possible to predict when or where blue-green blooms will occur. Although blooms can be observed somewhere in Washington almost any time of year, most occur in July through October (Hamel, 2009). Fish collection for the present study will focus on this period.

PDS-TSU will work with local health departments to identify lakes with blue-green blooms. PDS-TSU will alert the EA Program when high levels of microcystins are found or if saxitoxin is detected in Waughop Lake. The EA Program will mobilize to collect fish samples or make a request to WDFW that fish be collected.

Fish appear to take up microcystins quickly (Magalhães et al., 2001: Tencala and Dietrich, 1997). Depuration (loss) is also reported to be rapid once fish are removed from exposure (Adamovsky et al., 2007). Therefore, an attempt will be made to collect fish soon after high toxin levels are reported.

Five to ten individuals each of up to three fish species will be collected from lakes with significant blooms. Where possible, a range of feeding types will be retained to help identify species with a propensity toward toxin accumulation. Popular sport fish species such as trout, bass, and perch, as well as a bottom feeding species will be targeted. After all the fish

collections are completed – tentatively by the end of October – a decision will be made on which samples to analyze, in consultation with PDS-TSU and WDOH.

The budget for this project allows for analyzing microcystins in 20 fish tissue samples and saxitoxin in 5 fish tissue samples (not including quality control samples). Ten microcystin blooms will tentatively be sampled, assuming an average of two fish species collected for each bloom event. Fish for saxitoxin analysis will be collected on two occasions from Waughop Lake.

Because human health concern is the impetus for the study, all tissue samples will be fillets. Composite samples will be analyzed to provide a cost-effective estimate of mean toxin concentrations. Each sample will consist of composite of fillets from three or more individual fish.

Sediment Survey

Because microcystins are resistant to degradation they have the potential to persist in lakes long after a bloom has past. Babica et al. (2006) report detection of microcystins in freshwater sediments and cite several other studies with similar findings. Overwintering in freshwater sediments has been documented for microcystins (Ihle et al., 2005). Although similar concerns extend to saxitoxin, the present study will be limited to screening fish tissue for saxitoxin as a first step toward determining if a problem exists.

Ecology's algae monitoring data suggest that microcystins can dissipate fairly quickly, but the extent to which this is due to flushing, sedimentation, or other processes is unknown. If deposition is an important pathway, then sediments could act as a reservoir for uptake by fish or toxic effects to benthic organisms. Recreational exposure is a potential concern where algae surface scums wash up on beaches. In view of these considerations, lake sediments and beaches will be screened for the presence of microcystins.

PDS-TSU has identified five lakes - Waughop, Spanaway, Ketchum, Cassidy, and Anderson - that have a severe history of microcystin blooms and has recommended them for sediment sampling: Three composite sediment samples will be collected from each lake: one each from the deepest area, a shallow water area, and a beach or shoreline area. Deepwater, being relatively cold and dark, is assumed to have the greatest potential for toxin persistence, grading to beaches/shoreline which are exposed to full sunlight. Babica et al. (2006) observed higher microcystin concentrations in deepwater sediments and concluded that conditions were better for overwintering of cyanobacterial cells.

The sediments will be collected during the fall in view of seasonal trends reported in other studies on microcystins in sediment (Babica et al., 2006; Ihle et al., 2005). Selection of the sites for beach/shoreline samples will take into account the direction of prevailing winds during the summer bloom season which would tend to push blue-greens to a particular part of a lake.

Chemical Analysis

Microcystins

The fish samples from Ecology's 2008 study were analyzed through the courtesy of Dr. John Berry of Florida International University (FIU) using ELISA. In this assay, microcystins in a sample compete for a limited number of antibody binding sites located in small wells on a microtiter plate. After a wash and color development step, the plates are scanned with a spectrophotometer and the toxin concentrations quantitated by comparing optical densities to a calibration curve.

The ELISA-derived microcystin concentrations reported by FIU were generally consistent with concentrations reported in other fish tissue studies. However, matrix spike recoveries were low and precision of duplicate analyses was sometimes poor, raising questions about the quality of the data. Other studies, including those conducted by FIU, have achieved better results with ELISA (e.g., Wilson et al., 2008; Babica et al, 2006).

LC/MS is now considered to be the procedure of choice for analyzing cyanotoxins in a variety of environmental media (Bogialli et al., 2005; Lawrence and Menard, 2001.) CDFG recently developed and validated an enhanced LC/MS method (liquid chromatography-electrospray ionization tandem mass spectrometry) for analyzing microcystins in water and tissue (Mekebri et al., 2009). Six microcystin variants and nodularin (NDLN, also a blue-green hepatotoxin) are quantified (Table 5). The L, R, Y, A, F, and W designations stand for the variable amino acids leucine, arginine, tyrosine, alanine, phenylalanine, and tryptophan, respectively. Microcystin-LR and -YR are the most common toxins associated with blue-green blooms.

Table 5. Microcystin Variants and Nodularin Analyzed by the CDFG Method.

Toxin
MC-LR
MC-RR
MC-YR
MC-LA
MC-LF
MC-LW
NDLN

Using this method, Mekebri et al. report 80-104% recovery of all tested microcystins in fish tissue, with a percent relative standard deviation of <15% (n=8). The method detection limit is approximately 1 ug/Kg (parts per billion).

The present study proposes to use the CDFG LC/MS method to obtain verifiably accurate data on microcystins in fish tissue and sediment. These data will additionally be used to evaluate the accuracy of ELISA on the same set of fish tissue samples. The ELISA analysis is simpler, quicker, and less expensive than LC/MS. It is hoped that ELISA can be relied on for the Phase II study.

Several method comparison studies have been conducted for microcystins with ELISA and LC/MS or ELISA and HPLC (Bruno et al., 2006; Babica et al., 2006, Rapala et al., 2002; Deblois et al., 2008; Orr et al., 2003; Metcalf et al., 2000). Although good agreement has been achieved, ELISA sometimes underestimated or overestimated total microcystin concentrations due to different sensitivities to some toxic analogues or cross-reactivity with detoxification products, for example in liver tissue.

Unlike LC/MS, the results of an ELISA test are congener independent and give only the total concentration of microcystins in a sample. Both methods measure free forms of the toxin as opposed to bound forms. Although there is evidence that bound microcystins predominate in fish tissue, the free form is generally assumed to be more bioavailable and therefore the greater human health concern (Williams et al., 1997; Smith and Boyer, 2009).

Interactions between microcystins and inorganic (clay) particles seem to substantially affect the absorption of microcystins onto sediments. The toxin structure is another important factor that influences sorption onto sediments, with lower recoveries obtained for more hydrophilic variants, such MC-RR (Babica et al., 2006). ELISA gives a total value, and cannot differentiate between toxin variants that may be extracted with more or less success. Therefore, the sediment analysis for the present project will focus on LC/MS, which has the ability to identify individual toxin variants.

Saxitoxin

The fish samples will be analyzed for saxitoxin by the ELISA method which was originally developed for use on shellfish tissue. Sediment samples will not be analyzed for saxitoxin, as previously mentioned.

Sampling Procedures

Fish

Fish will be collected by electroshocking, gill net, fyke net, or hook and line, following the EA Program SOP (Sandvik, 2006a). Only legal size fish will be taken for analysis, where size limits apply. For species with no size limits, only those large enough to reasonably be retained for consumption will be taken.

Fish selected for analysis will be killed by a blow to the head. The fish will be put in new plastic bags, and placed on ice as soon after collection as possible. The fish will be transported to Ecology headquarters on ice or frozen if transport is delayed by more than two days.

At Ecology headquarters each fish will be given a unique identifying number and its length and weight recorded. The fish will be individually wrapped in aluminum foil, put in plastic bags, and frozen pending preparation of tissue samples.

Tissue samples will be resected at Ecology headquarters following the EA Program SOP (Sandvik, 2006b). Techniques to minimize potential for sample contamination will be used. People preparing the samples will wear non-talc nitrile gloves and work on heavy duty aluminum foil or a polyethylene cutting board. The gloves and foil will be changed between samples; the cutting board will be cleaned between samples as described below.

The fish will be thawed enough to remove the foil wrapper and rinsed with tap water, then deionized water to remove any adhering debris. The fish will be scaled, except for bullheads and catfish which are eaten without the skin. The entire fillet from one or both sides of each fish will be removed with stainless steel knives and homogenized in a Kitchen-Aid blender.

Liver weights will be recorded after filleting. Mohamed et al. (2003) reported an increase in liver to body weight ratio with increased fish exposure “showing the presence of liver enlargement induced by MCs”.

All tissues will be homogenized to uniform color and consistency. The homogenates will be placed in 4 oz. glass jars that have been cleaned to EPA (1990) QA/QC specifications. Each tissue homogenate will be split into two glass jars, one each for CDFG and KCEL.

Cleaning of resecting instruments, cutting boards, and blender parts will be done by washing in tap water with Liquinox detergent, followed by sequential rinses with tap water, de-ionized water, and pesticide-grade acetone. The items will then be air dried on aluminum foil in a fume hood before use.

The tissue samples will be refrozen for overnight shipment with chain-of-custody record to the analyzing laboratories. The samples will be maintained at or near freezing during shipment.

Sediment

Sediment sampling methods will follow EA Program standard operating procedures (Blakley, 2008). The samples will be collected using a 0.02 m^2 or 0.05 m^2 stainless steel Ponar grab. A grab will be considered acceptable if not over-filled with sediment, overlying water is present and not excessively turbid, the sediment surface is relatively flat, and the desired depth penetration has been achieved. A field log will be maintained during sampling to record date, time, GPS coordinates, water depth, grab penetration depth, and description of the material obtained. Sampling sites will be located and positions recorded using GPS and landmarks.

All samples will be composites of the top 2 cm layer, consistent with routine practice for surveys of chemical contaminants in surface sediments (Ecology, 2008). After siphoning off overlying water, the top 2 cm of sediment from each of three grabs per sampling site will be removed with a stainless steel scoop, placed in a stainless steel bowl, and homogenized by stirring. Material touching the side walls of the grab will not be taken.

Subsamples of the homogenized sediment will be put into 4 oz. glass jars that have been cleaned to EPA (1990) QA/QC specifications and placed on ice immediately upon collection. The samples will be returned to Ecology HQ and held frozen until transport with chain-of-custody record to CDFG.

Stainless steel implements used to collect and manipulate the sediments will be cleaned by washing with Liquinox detergent, followed by sequential rinses with tap water, deionized water, and pesticide-grade acetone. The equipment will then be air dried and wrapped in aluminum foil. Between-sample cleaning of the Ponar at each lake will consist of thorough brushing with on-site water.

Measurement Procedures

Table 6 summarizes measurement procedures for this study.

Table 6. Measurement Procedures.

Analysis	Number of Samples*	Expected Range of Results	Reporting Limit	Sample Extraction Method	Analytical Method
Fish Tissue					
Microcystins by LC/MS	22	<1 - 100 ug/Kg	1 ug/Kg	methanol-water	LC/MS (Mekebri et al., 2009)
Microcystins by ELISA	22	<1 - 100 ug/Kg	1 ug/Kg	methanol-water	ELISA (Envirologix)
Saxitoxin by ELISA	6	<10 ug/Kg	1 ug/Kg	methanol-water	ELISA (Abraxis)
Sediment					
Microcystins by LC/MS	17	unknown	TBD	methanol-water	LC/MS (Mekebri et al., 2009)

*includes reference lake samples.
TBD: to be determined.

The LC/MS method proposed for this project is not accredited. Therefore, a waiver for its use has been requested from the Ecology QA Officer (6/22/2010).

Microcystins by LC/MS

The method to be used for microcystins in tissue and sediment is described in Mekebri et al., (2009) as applied to water and tissue. Briefly, 2-5 g tissue samples are transferred to centrifuge tubes with 10 mL methanol:acidified water (90:10, v/v) and finely-ground, followed by sonication. The extract is reduced in volume, diluted with water, acidified, and cleaned-up using solid phase extraction (SPE). Analysis is by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS).

For the present study, MC-RR, -LR, -YR, -LA, - desmethyl LR, and nodularin will be quantified since standards are currently available for these variants. The samples will also be screened for MC-LW, -LF, and - desmethyl RR.

The tissue data will be reported on a wet weight basis. The sediment data will be reported on a dry weight basis.

Method Validation for ELISA

For this project, KCEL will be modifying the ELISA methods they currently use on the Algae Control Program's bloom samples. KCEL is therefore initiating a validation study for analyzing microcystins and saxitoxin in tissue. It will include a 3-day, 7 replicate MDL study. A minimum of 2 sets of spike blanks and MS/MSD will be analyzed. QC sample control limits for the tissue matrix will be evaluated and updated as necessary. For microcystin, KCEL will tentatively use a methanol-water extraction followed by SPE cleanup, similar to the extraction in the Mekebri et al. method.

The results of the validation study will be summarized in a memorandum from KCEL and included as an addendum to this QA Project Plan.

Microcystins Analysis by ELISA

The analysis of microcystins in fish tissue by ELISA will be run in accordance with the Envirologix kit insert and KCEL SOP 440v2 as modified for the tissue matrix. The ELISA test kit uses polyclonal antibodies that bind either microcystin 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 microcystin allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of microcystin 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 (Table 7).

The data will be reported on a wet-weight basis.

Table 7. Microcystin Cross-reactivity.

Toxin Variant	Sensitivity/ Cross Reactivity [1,2]
Microcystin - LR	100%
Microcystin - LA	62%
Microcystin - RR	54%
Microcystin - YR	35%
Nodularin	68%

[1] For example, Nodularin is detected at 68 % of the actual dose.
[2] Source: Larrivee (2010).

Saxitoxin Analysis by ELISA

The saxitoxin ELISA of tissue will be run in accordance with the Abraxis kit insert and KCEL SOP 462v0 modified for the tissue matrix. The test is a direct competitive ELISA based on the recognition of saxitoxin by specific antibodies. Saxitoxin, when present in a sample and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. The plate kit recognizes saxitoxin and other PSP toxins with varying degrees (Table 8).

Table 8. Saxitoxin Cross-reactivity.

Toxin Variant	Sensitivity/ Cross Reactivity [1]
STX	100%
Decarbamoyl STX	29%
GTX 2 and 3	23%
GTX 5B	23%
Sulfo GTX 1 and 2	2.0%
Decarbomyl GTX 2 and 3	1.4%
Neosaxitoxin	1.3%
Decarbomyl Neo STX	0.6%
GTX 1 and 4	<0.02 %

[1] Abraxis, 2010.

The data will be reported on a wet-weight basis.

Quality Control Procedures

Field

Fish tissue and sediment samples from two reference lakes will be submitted to the analyzing laboratories as a check against false positives in the microcystin and saxitoxin analyses. Oligotrophic lakes located away from developed areas and without any history of algae blooms will be selected. Reference lakes for this study have not yet been identified.

Laboratory

Laboratory QC samples to be used in assessing the precision and bias of data obtained through this project are shown in Table 9. The samples for duplicate analysis will be identified by the project lead.

Reference materials will be incorporated as available.

Table 9. Laboratory Quality Control Procedures.

Analysis	Method Blanks	Spiked Blanks	Check Std/ Positive Control	Analytical Duplicates	Surrogate Spikes	MS/ MSD
Fish Tissue						
Microcystins by LC/MS	1/batch	1/batch	1/batch	2	all samples	1/batch
Microcystins by ELISA	1/batch	1/batch	1/batch	2	NA	1/batch
Saxitoxin by ELISA	1/batch	1/batch	1/batch	1	NA	1/batch
Sediment						
Microcystins by LC/MS	1/batch	1/batch	1/batch	2	all samples	1/batch

MS/MSD: matrix spike and matrix spike duplicate.
NA: not applicable.

Reference materials will be incorporated as available.

Laboratory Cost Estimate

Table 10 has an estimate of laboratory costs for this project. The EA Program Toxics Studies Unit will arrange for sample analysis through an interagency agreement with CDFG and through an amendment to the existing algae control project contract between KCEL and WQP.

Table 10. Laboratory Cost Estimate.

Analysis	Matrix	Method	Field Samples	QC Samples*	Cost per Sample	Cost Subtotals
Microcystins	Tissue	LC/MS	20	4	$454	$10,442
		ELISA	20	4	$118	$2,832
	Sediment	LC/MS	15	4	$323	$5,814
Saxitoxin	Tissue	ELISA	5	2	$312	$2,184
					LC/MS data review by Manchester =	$1,320
					Total Cost =	$22,592

*Reference lake samples and laboratory duplicates (one duplicate is not charged).

Data Management Procedures

Field data, including length/weight data on the fish samples, will be recorded in a bound notebook of waterproof paper. These data will be transferred to Excel spreadsheets and verified for accuracy.

The laboratories will provide a case narrative discussing any problems with the analyses, corrective actions taken, changes to the referenced method, and an explanation of data qualifiers. The lab data package should also include all QC results associated with the data. This information is needed to evaluate the accuracy and to determine whether the MQOs were met. The narrative should include results for all blanks, check standards/laboratory control samples, reference materials, and surrogates included in the sample batch, as well as results for analytical duplicates and matrix spikes.

Data Verification

The Ecology Manchester Environmental Laboratory (MEL) will conduct a review of the LC/MS data for this project. MEL will verify that methods and protocols specified in this QA Project Plan were followed; that all calibrations, checks on quality control, and intermediate calculations were performed for all samples; and that the data are consistent, correct, and complete, with no errors or omissions. Evaluation criteria will include the acceptability of instrument calibration, procedural blanks, check standards, recovery and precision data, and appropriateness of any data qualifiers assigned. MEL will prepare written data verification reports based on the results of their review. A case summary can meet the requirements for a data verification report.

Review of the ELISA data will follow standard PDS-TSU procedures for the Freshwater Algae Control Program.

The project lead will review the laboratory data packages and data verification reports. To determine if project MQOs have been met, results for check standards/positive controls, duplicate samples, reference materials (as available), surrogates, and matrix spikes/duplicates will be compared to QC limits. Method blank results will be examined to verify there was no significant contamination of the samples. To evaluate whether the targets for reporting limits have been met, the results will be examined for non-detects and to determine if any values exceed the lowest concentration of interest.

Based on these assessments, the data will be either accepted, accepted with appropriate qualifications, or rejected and re-analysis considered.

Data Analysis

Once the data have been verified, the project lead will determine if they can be used to make the determinations for which the project was conducted. If the MQOs have been met, the quality of the data should be useable for meeting project objectives and report preparation will proceed.

Data from the Freshwater Algae Control Program will be downloaded and summarized to assess bloom conditions preceding sample collections. The quality of the fish tissue and sediment data will be evaluated and any shortcomings in its usefulness identified. Summary statistics will be calculated and graphical displays of the data prepared. Correlation analysis will be applied to the LC/MS and ELISA fish tissue data. The fish and sediment data will be compared to results from Ecology's 2008 study and similar studies done elsewhere.

Audits and Reports

Audits

Laboratory audits will not be conducted for this study.

Reports

The fish and sediment data will be provided to Dr. Joan Hardy, Office of Environmental Health, Safety, and Toxicology of WDOH for her use in assessing human health risk.

A draft project report will be prepared for review by the client and WDOH. The tentative date for this report is May 2011. A final technical report is anticipated in July 2011. The responsible staff member is Art Johnson.

The draft report will include:

- Maps of the study area.
- Descriptions of each lake where samples were analyzed.
- Descriptions of field and laboratory methods.
- Length and weight data for the fish samples.
- Data on bloom conditions surrounding sample collection.
- Discussion of data quality.
- Summary tables and graphical displays of the chemical data.
- Correlation between LC/MS and ELISA.
- Comparisons with results from 2008 and similar studies done elsewhere.
- Recommendations for the Phase II study.

The project data will be entered into Ecology's Environmental Information Management System (EIM) on or before July 2011. The responsible staff member is Michael Friese.

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Appendix. Acronyms, Abbreviations, and Units of Measurement

CDFG	California Department of Fish and Game
EA Program	Ecology Environmental Assessment Program
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
ELISA	Enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
GPS	Global Positioning System
HPLC	High pressure liquid chromatography
KCEL	King County Environmental Laboratory
LC/MS	Liquid chromatography/mass spectrometry
MEL	Ecology Manchester Environmental Laboratory
MQO	Measurement quality objective
PDS-TSU	Program Development Services Section, Technical Services Unit
QA	Quality assurance
QC	Quality control
RPD	Relative percent difference
SOP	Standard operating procedures
WDFW	Washington Department of Fish and Wildlife
WDOH	Washington State Department of Health

Units of measurement

ug/Kg	micrograms per kilogram (parts per billion)
µg/L	micrograms per liter (parts per billion)