Quality Assurance Project Plan

Screening for Blue-Green Algae Toxins in Lake Fish Tissues

by
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October 2008

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

Table of Contents

Abstract

Each study conducted by the Washington State Department of Ecology must have an approved Quality Assurance Project Plan. The plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After study completion, a final report describing the results will be posted to the Internet.

In the present study, approximately 50 fish tissue samples from several Western Washington lakes will be screened for the blue-green algae toxins microcystin and anatoxin-a. These compounds can affect the liver and nervous system of animals, including humans, and are an emerging public health issue. Until now, the primary exposure pathways of concern have been through drinking water and recreational exposure. Consumption of fish containing blue-green algae toxins represents a poorly studied but potentially important exposure route for humans.

Up to 36 fish tissue samples will be analyzed for microcystins and up to 10 samples for anatoxin-a, depending on where blooms occur and their severity. Most of the samples will be fillets, with a limited number of liver samples. Blue-green bloom activity will be monitored through the summer and early winter of 2008 by local health departments and the Ecology Water Quality Program. Fish samples will be collected by the Washington Department of Fish and Wildlife. The data will be provided to the Washington State Department of Health for their use in assessing the potential human health concern.

Background[1]

Cyanobacteria, commonly known as blue-green algae, are bacteria that contain photosynthetic pigments similar to those found in algae and plants. Their ability to fix nitrogen gives them a competitive advantage over other algae. Many blue-greens have gas vacuoles that keep them near the surface where there is more light for photosynthesis. Colonies may clump together and form a surface scum which causes water quality problems in lakes.

Blue-greens can produce powerful toxins that affect the liver and nervous system of animals, including humans. The toxins of interest in the present study are microcystins and anatoxin-a. Microcystins primarily affect the liver and can promote liver tumors. Anatoxin-a affects nerve synapses. Some of these compounds (especially microcystins) are very stable and can remain in the water for days or weeks after a bloom has disappeared, while anatoxin-a is less stable.

Figure 1. Blue-green Algae.

The blue-green genera *Microcystis* and *Anabaena* cause water quality problems in Washington lakes and can produce microcystins and anatoxin-a. *Anabaena spp.* can produce several kinds of toxins, including anatoxin-a, anatoxin-a(s), saxitoxin, and microcystins. (Anatoxin-a(s) has a different structure and mode of action than anatoxin-a, and is thought to be relatively uncommon). Some *Microcystis* species produce microcystins. They, along with *Aphanizomenon*, account for the vast majority of blue-green blooms in Washington. A bloom can consist of one or a mixture of two or more genera of blue-greens.

Most blue-green blooms occur during the summer. However, toxic blooms can also occur during the winter. American Lake in Pierce County has a history of toxic episodes during the winter at low water temperatures (7-8^{o}C). A bloom of blue-green algae can potentially be found somewhere in Washington nearly any month of the year. Factors needed for a bloom to occur are complex. No individual environmental cause or particular set of conditions clearly controls their formation. Even blooms caused by known toxin producers may not produce toxins or may produce toxins at undetectable levels.

Blue-greens cannot maintain an abnormally high population for long and will rapidly die and disappear after 1-2 weeks. If conditions remain favorable, another bloom can replace the previous one in such a way that it may appear as if one continuous bloom lasts for up to several months.

[1] The above information is from the Washington State Department of Health, Office of Environmental Health Assessments cyanobacteria web page. Figure 1 photograph by Gene Williams, Snohomish County Public Works. www.doh.wa.gov/ehp/algae/whatarecyanobacteria.htm

Toxic blue-greens are an emerging public health issue (Stone and Bress, 2007). The primary exposure pathways of concern have been through drinking water and recreational exposure. Consumption of fish containing blue-green toxins represents a poorly studied but potentially important exposure route for humans (Stone and Bress, 2007; Kann, 2008; Wilson et al., in press). Microcystins, for example, are heat stable and do not break down during cooking (Harada et al., 1996). Researchers suspect these compounds are liver carcinogens, which could prove significant to humans following continuous, low-level exposure. Anatoxin-a is less stable than microcystins and, at present, more difficult to analyze.

Freshwater and brackish-water fish are known to accumulate cyanotoxins in their tissues, including muscle, viscera, and liver (Kotak et al., 1996; Sipiä et al., 2001; Ibelings et. al., 2005; Gkelis et. al., 2006; Wood et. al., 2006). Concentrations of microcystins are routinely shown to be much higher in liver versus other tissues (Magalhães et. al., 2001; Xie et. al., 2005; Chen et. al., 2007; Zhao et. al., 2006).

Microcystins have been found in yellow perch muscle from Lake Erie (Wilson et al., in press). Concentrations were in the range of 0.12 – 4.0 ug/Kg, dry (parts per billion). Much higher concentrations were measured in liver, 17-1,182 ug/Kg, dry. The yellow perch sampling period coincided with a massive cyanobacterial bloom where *Microcystis aeruginosa* was the dominant species. In this study, the levels of microcystins did not appear to be a human health concern.

Microcystins in fish tissue have, however, been identified as a potential human health concern in two Klamath River reservoirs (Kann, 2008). That study concluded that yellow perch muscle and freshwater mussels exceeded total daily allowable intake guidelines for microcystins, based on a review by Ibelings and Havens (2007). A public health advisory was recommended.

In light of the known uptake of blue-green toxins by fish and the potential for adverse human health effects, the Program Development Services Section, Technical Services Unit (PDS-TSU) of Ecology's Water Quality Program has requested that fish samples from selected Western Washington lakes be screened for microcystins and anatoxin-a during or following blue-green blooms.

Project Description

This project will analyze the blue-green algae toxins microcystin and anatoxin-a in food fish tissues collected in association with algal blooms in selected Western Washington lakes during the summer and early winter of 2008. The lakes and species sampled will depend on where significant blooms occur and their severity. The objective will be to obtain screening-level data that can be used to assess the potential human health concern from fish consumption. The trigger for sampling will be high levels of microcystins or anatoxin-a in water samples collected by local health departments: www.ecy.wa.gov/programs/wq/plants/algae/monitoring/index.html.

The health departments and the Ecology Water Quality Program will monitor bloom conditions in local lakes to determine when and where fish samples should be collected. The Ecology Environmental Assessment (EA) Program will be notified of significant blooms and will contact the Washington Department of Fish and Wildlife (WDFW). WDFW will collect fish samples for analysis, as their schedule allows.

Extracts from the fish tissue samples will be analyzed by Florida International University (microcystins) and the King County Environmental Laboratory (anatoxin-a). Up to 36 microcystin samples and 10 anatoxin-a samples will be analyzed, focusing on edible tissues.

The EA Program will lead the study and prepare a project report. The data will be provided to the Washington State Department of Health for their use in assessing human health concern.

Organization and Schedule

The following people are involved in this project.

Table 1. Organization of Project Participants and Responsibilities.

Staff	Title	Responsibilities
Art Johnson Toxics Studies Unit Statewide Coordination Section, EAP, Ecology (360) 407-6766	Principal Investigator	Writes the QAPP, coordinates fish collections by WDFW and chemical analyses, conducts QA review of data, analyzes and interprets data, writes the draft report and final report.
Dale Norton Toxics Studies Unit Statewide Coordination Section, EAP, Ecology (369) 407-6765	Unit Supervisor	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Will Kendra Statewide Coordination Section, EAP, Ecology (360) 407-6698	Section Manager	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Kathy Hamel PDS-TSU Water Quality Program, Ecology (360) 407-6562	EAP Client	Clarifies scopes of the project, provides internal review of the QAPP, and approves the final QAPP.
Rich Eltrich Washington Dept. Fish & Wildlife (253) 589-7233	Fisheries Biologist	Contact for fish collection in American Lake.
Adam Couto Washington Dept. Fish & Wildlife (360) 902-8312	Fisheries Biologist	Contact for fish collections in other lakes.
John Berry Florida International Univ. (305)919-4569	Assistant Professor	Sample extractions, quality control sample preparation, and microcystin analyses.
Gabriela Hannach King County Environmental Laboratory (206) 684-2358	Aquatic Toxicologist	Anatoxin analyses.
William R. Kammin EAP, Ecology (360) 407-6964	Ecology Quality Assurance Officer	Reviews and approves the QAPP.
Joan Hardy Environmental Health Assessments WA. State Department of Health (360) 236-3173	Toxicologist	Health department contact for receiving project data.
Brandee Era-Miller Toxics Studies Unit Statewide Coordination Section, EAP, Ecology (360) 407-6771	Data Engineer	Enters project data in EIM.

EAP – Environmental Assessment Program
EIM – Environmental Information Management system
Ecology – Washington State Department of Ecology
PDS-TSU – Program Development Services, Technical Services Unit

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

Field and laboratory work	
Field work completed	December 2008
Laboratory analyses completed	March 2009
Environmental Information System (EIM) system	
EIM data engineer	Brandee Era-Miller
EIM user study ID	AJOH0058
EIM study name	Blue-green Toxins in Fish
Data due in EIM	July 2009
Final report	
Author lead	Art Johnson
Schedule	
Draft due to supervisor	May 2009
Draft due to client/peer reviewer	June 2009
Final report due on web	July 2009

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

Florida International University and King County Environmental Laboratory are expected to meet all QC requirements of the analytical methods being used for this project.

Measurement quality objectives (MQOs) for the analyses being conducted are shown in Table 3. The recovery and precision objectives are what the analyzing laboratories anticipate achieving on project samples. The lowest concentration of interest for microcystins is the detection limit of the method. The detection limit of the anatoxin-a method as applied to fish tissue has not been determined at this time.

Table 3. Measurement Quality Objectives for Blue-Green Toxins Screening.

Analysis	Check Stds./ Spike Blank (% recov.)	Duplicate Samples (RPD)	Surrogate Recovery (% recov.)	Matrix Spikes (% recov.)	Matrix Spike Duplicates (RPD)	Lowest Concentration of Interest (ug/Kg, wet)
Microcystin	80-120%	≤ 25%	NA	80-120%	≤ 25%	0.15
Anatoxin	50-150%	≤50%	TBD	50-150%	≤50%	5

LCS = laboratory control sample
RPD = relative percent difference
NA = not analyzed
TBD = to be determined

Sampling Design

This study will focus on local lakes in Western Washington. The Water Quality Program has identified 12 lakes in this region with a consistent history of blue-green blooms and thus of potential interest for the study (Table 4).

Table 4. Lakes of Potential Interest for Screening Blue-Green Toxins in Fish Tissue.

Lake	County	Lat	Long
American	Pierce	47.132°N	122.565°W
Spanaway	Pierce	47.110°N	122.446°W
Steilacoom	Pierce	47.117°N	122.440°W
Tanwax	Pierce	46.940°N	122.275°W
Waughop	Pierce	47.170°N	122.565°W
Anderson	Jefferson	48.020°N	122.801°W
Leland	Jefferson	47.897°N	122.882°W
Gibbs	Jefferson	47.973°N	122.814°W
Cassidy	Snohomish	48.053°N	122.094°W
Ketchum	Snohomish	48° 16' 56"	122° 20' 42"
Ward	Thurston	47.009°N	122.876°W
Sammamish	King	47.600°N	122.098°W

It is not possible to predict with certainty when or where blooms will occur. PDS-TSU will work with local health departments to identify lakes with blue-green blooms. Following routine practice, health department personnel will collect algae samples to determine the species involved and monitor the bloom's progress. These samples will be analyzed through an existing contract with the King County Environmental Laboratory. PDS-TSU will alert the EA Program when high levels of microcystins or anatoxin-a are detected. The EA program will then contact WDFW to request that fish samples be collected.

Concern about consumption of American Lake kokanee (land-locked sockeye salmon) was the original impetus for this study. Don Russell, a local property owner knowledgeable on the blue-green problem, initially raised the human health issue. For the past five years, WDFW has been working with the support of personnel from Camp Murray to establish a kokanee broodstock in American Lake. The lake now supports a popular kokanee sport fishery and has been chosen as a potential broodstock sanctuary for kokanee in the South Puget Sound region.

Kokanee feed on *Daphnia* and other zooplankton that may have accumulated toxins from phytoplankton in the lake. At the time of the lake's fall turn over, *Anabaena* blooms occur, fed by soluble phosphorus from the sediments and hypolimnion. The result is toxic *Anabaena* scums that form on the lake surface. During the fall of 2007, the scum was reported to contain 4,000 ug/L of anatoxin-a. (Don Russell, 5/16/2008 email).

For the present study, five to ten individuals each of one or two fish species will be collected from each lake where significant blooms are detected. Microcystins and anatoxin-a are not thought to transfer far up the food chain (John Berry, FIU, personal communication). Therefore herbivores, insectivores, and omnivores will be the preferred fish species for sampling.

The time required for uptake and depuration of these compounds is unknown. An attempt will be made to collect fish soon after high toxin levels are reported. Sample timing depends on WDFW's ability to fit this activity into their existing schedule. After all the fish collections are completed (tentatively December 2008), a decision will be made on which samples to analyze, based on PDS-TSU and WDOH assessments of the type and severity of the blooms.

The budget for this project allows for analyzing microcystins in up to 36 fish tissue samples and anatoxin-a in up to 10 samples. The analyses are being weighted toward microcystins because it is the more persistent compound and the focus of the recent literature. Additionally, anatoxin-a is a less stable compound than microcystins which may complicate quantification efforts.

Since human health concern is the impetus for the study, most of the tissue samples will be fillets. A limited number of liver samples will also be analyzed. Fillets will be analyzed from individual fish to provide estimates of variability. Liver will be analyzed as composite samples from up to five fish each.

Sampling Procedures

Fish will be collected by electroshocking, gill net, or hook and line. Only legal size fish will be taken for analysis. 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, 2006). 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 entire fillet from one or both sides of each fish will be removed with stainless steel knives and homogenized in a Kitchen-Aid blender. The fillets will be skinned as an additional step to avoid surface contaminants. After filleting, the body cavity will be opened and the liver removed and homogenized using a stainless steel sonicator device designed for preparation of small samples.

All tissues will be homogenized to uniform color and consistency. The homogenates will be placed in 2-4 oz. glass jars with Teflon lid liners, cleaned to EPA (1990) QA/QC specifications.

Cleaning of resecting instruments, cutting boards, blender, and sonicator 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 shipment with chain-of-custody record to Dr. John Berry, Florida International University (FIU), North Miami, Florida.

Laboratory Procedures

Laboratory procedures for this project are shown below, along with the anticipated number of samples, expected range of results, and reporting limits of the methods.

Table 5. Laboratory Procedures for Blue-Green Toxins Screening.

Analysis	Number of Samples	Expected Range of Results*	Reporting Limit (wet wt.)	Sample Extraction Method	Analytical Method
Microcystins	36	<0.15 - 5 ug/Kg	0.15 ug/Kg	methanol	ELISA (Envirologix)
Anatoxin-a	10	unknown	5 ug/Kg	methanol/SPE	James et al. (1998)

*muscle tissue
SPE = solid phase extraction

Florida International University (FIU) will perform separate extractions of the tissue samples for microcystins and anatoxin-a. FIU will ship the dry, frozen anatoxin-a extracts (i.e., post acidic methanol and SPE) to Gabriela Hannach of the King County Environmental Laboratory, Seattle.

Aliquots of the tissue samples to be extracted for microcystins will be freeze-dried and weighed. Microcystins will be extracted twice from each sample using sequentially 75% aqueous methanol and 75% aqueous with 0.4% acetic acid. Extracts will be collected via centrifugation, glass-fiber filtered, dried using vacuum evaporation, and redissolved in water using sonication.

FIU will analyze the microcystin extracts by enzyme-linked immunosorbent assay (ELISA) using commercially available kits as per the procedure described by the manufacturer (Envirologix EP022 http://envirologix.com/artman/publish/index.shtml). The results, uncorrected for recovery rates, are for the freely available toxin and do not include covalently-bound microcystins. The concentrations are expressed as microcystin-LR equivalents, based on the ELISA protocol.

At FIU, anatoxin-a will be extracted three times in acidic methanol (1% HCl 1 M), followed by weak cation exchange solid-phase extraction (SPE) for clean-up, as per James et al. (1997). Dry anatoxin-a extracts will be shipped frozen to King County. Upon arrival, extracts will be reconstituted prior to analysis by HPLC. The anatoxin method King County currently uses on water samples will be adapted if necessary for use on tissue. In this method, anatoxin-a is converted into a fluorescent derivative and detected by isocratic HPLC-FD. FIU will provide King County with spiked and non-spiked tissue extracts for method validation prior to the start of the project.

The FIU tissue samples will be approximately 3 grams wet weight. King County Environmental Laboratory has estimated that the following tissue weights will be necessary for the anatoxin-a analysis:

Sample/QC Type	Wet Wt. Tissue (g)
Sample A	3 g
Sample A MS	3 g
Sample A MSD	3 g
Total QC Sample A	9 g
Sample B	3 g
Sample B Duplicate	3 g
Total QC Sample B	6 g
All other samples	3 g

The laboratory cost estimate for this project is $3,500. Microcystins are being analyzed at no cost by FIU. Ecology will reimburse FIU for the cost of the ELISA plates used in the analysis (~$800). King County will analyze the anatoxin extracts under their current Dept. of Ecology Algae Control Program contract at $150/sample. The laboratory cost estimate includes 10% for split samples and a 25% surcharge for data review by Manchester Laboratory. Data review by Manchester is tentative until they can complete an initial review of the analytical methods.

Quality Control Procedures

Field

No field Quality Control samples are planned for this project.

Laboratory

Table 6. Laboratory Quality Control Procedures for Blue-Green Toxins Screening.

Analysis	Method Blanks	Check Stnds/ Spike Blanks	Analytical Duplicates	Surrogate Spikes	MS/MSD
Microcystins	2/batch	1/batch	4	NA	1/batch
Anatoxin-a	1/batch	1/batch	2	TBD	1/batch

LCS = laboratory control sample
MS/MSD = matrix spike and matrix spike duplicate
NA = not analyzed
TBD = to be determined

Method blanks, spike blanks, laboratory duplicates, matrix spikes, and matrix spike duplicates must be processed through all steps of preparation and analysis.

Data Management Procedures

Field data and 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 chemical data will be reported on a wet weight basis. FIU will send King County the sample weights of individual extracts so that the anatoxin-a tissue concentrations can be calculated.

Data Verification

Manchester Laboratory will conduct a review of all laboratory data for this project (tentative). Manchester will verify that (1) methods and protocols specified in this Quality Assurance Project Plan were followed; (2) all calibrations, checks on quality control, and intermediate calculations were performed for all samples; and (3) 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. Manchester 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.

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, lab control samples, duplicate samples, surrogates, and matrix spikes 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 Quality (Usability) Assessment

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.

The health risk assessment for these data will be conducted by WDOH. Therefore, the data analysis conducted for the project report will be limited. The report will assess the quality of the fish tissue data and identify any shortcomings in their usefulness. Summary statistics and graphical displays of the results will be provided as appropriate. Data from the local health departments describing bloom conditions preceding sample collections will be summarized. The fish tissue data will be compared to results of similar studies done elsewhere, as available.

Audits and Reports

Audits

Laboratory audits will not be conducted for this study.

Reports

The fish tissue data will be provided to WDOH as it is received.

A draft project report will be prepared for review by the client, WDOH, WDFW, and stakeholders. The tentative date for this report is May 2009. A final technical report is anticipated in July 2009. The responsible staff member is Art Johnson.

The draft report will include:

- maps of the study area
- descriptions of each lake where fish samples were analyzed
- concentrations of blue-green toxins reported in the lakes prior to fish collection
- descriptions of field and laboratory methods
- discussion of data quality and the significance of any problems encountered in the analyses
- summary tables and graphical displays of the chemical data
- comparisons with results of similar studies
- recommendations

The project data will be entered into Ecology's Environmental Information Management (EIM) System on or before July 2009. The responsible staff member is Brandee Era-Miller.

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List of Acronyms

Following are acronyms and abbreviations used frequently in this report.

EAP	Environmental Assessment Program
Ecology	Washington State Department of Ecology
FIU	Florida International University
MQO	Measurement Quality Objectives
PDS-TSU	Program Development Services - Technical Services Unit
QA	Quality Assurance
QC	Quality Control
WDFW	Washington Department of Fish and Wildlife
WDOH	Washington State Department of Health