Comparison of Toxicity in Lake Union Sediments Using Molecular DNA Techniques and Conventional Bioassay Measures

Quality Assurance Project Plan

By
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Background and Problem Statement

Lake Union is a heavily urbanized watershed. The lake has been substantially altered through shoreline filling and by the dredging of two channels, one into Lake Washington and one into Salmon Bay. Water levels in the Lake Washington/Lake Union system have been altered due to the disruption of the Black River in Renton and salinities vary due to the operation of the Ballard Locks. Despite these activities changing Lake Union from a spring-fed lake into a regional waterway, salmon migrate through Salmon Bay and Lake Union.

Gas Works Park (GWP) is a 20-acre city park located on the north shoreline of Lake Union in Seattle, Washington (Figure 1). Industrial facilities were developed on the site in 1903, and coal gasification began in 1906. In 1956, the Seattle Gas Company ceased operations when natural gas became available, and in 1962 the facility was sold to the City of Seattle. Wastes found at the site include solvent-soaked wood chips, slag, lampblack carbon, coal by-products, and tar.

Studies published by the Washington State Department of Ecology (Ecology) and the United States Environmental Protection Agency (USEPA) have documented sediment contamination in Lake Union (Hileman et al., 1985; Yake et al., 1986; Cubbage, 1992). Analysis of sites along the north shore of Lake Union in the vicinity of Gas Works Park found high levels of polycyclic aromatic hydrocarbons (PAHs); polychlorinated biphenyls (PCBs); and elevated concentrations of arsenic, copper, lead, zinc, and other metals.

A number of other agencies have also collected sediment chemistry and bioassay data in Lake Union. These data (Tomlinson et al., 1977; Hansen, 1993; TAMU, personnel communication) were reviewed, in conjunction with Ecology's statewide database of sediment chemistry and bioassay data, SedQual.

This study is intended to determine the nature and extent of sediment toxicity using conventional bioassays. The selected sites may also compare conventional bioassay mortality and growth endpoints with deoxyribonucleic acid (DNA) mutagenic analysis across PAH contaminant gradients.

Project Description

This project is a cooperative effort between Ecology, Texas A&M University (TAMU), and the USEPA. Both Ecology and TAMU will conduct field and analytical analyses, while USEPA provides coordination and oversight. This cooperative study is funded through an USEPA grant to TAMU.

Ecology's Environmental Assessment Program (EAP) will analyze sediments using bioassays at eleven sites across northern Lake Union to determine the nature and extent of sediment contamination associated with prior coal gasification activities nearby. Two reference sites on Lake Washington will also be sampled. The 11 Lake Union sites are located across the projected gradient of contaminant concentrations to attempt to describe the magnitude and limits of toxic effects associated with the site. The methods described in this QAPP address sediments collected by EAP for toxicity evaluations using *Hyalella* and *Chironomus* bioassays.

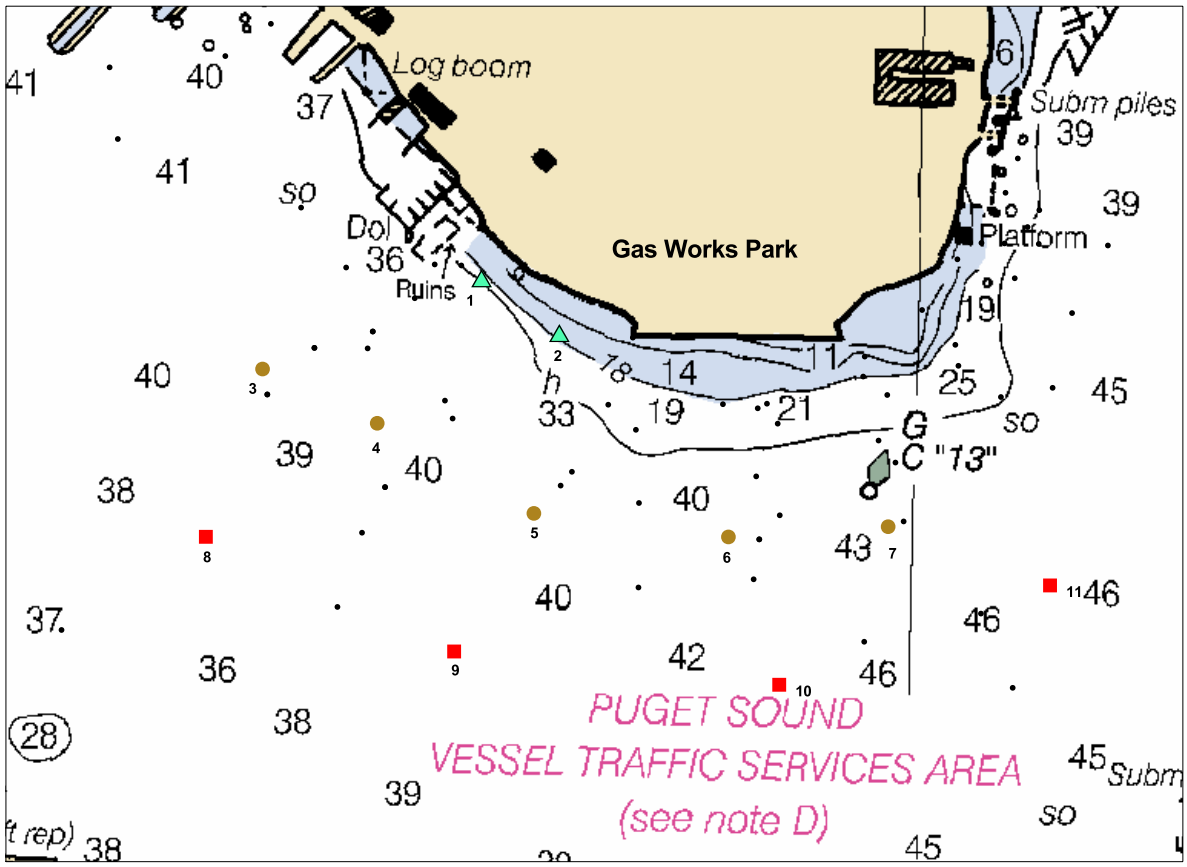

Figure 1. Lake Union, WA Sediment Bioassay Stations by Contaminant Zone

Ecology does not propose chemical analysis in this project plan, although EAP will collect the sediment to be used in chemical and Microtox® analyses by TAMU. TAMU will analyze all of the EAP sites for a selected list of metals and organic compounds (Table 1). In addition to the chemical analysis, TAMU will also conduct Microtox® bioassays at each of the Ecology's stations.

TAMU will be developing new methods for measuring biomarkers of mutagenic changes in Coho salmon DNA (Donnelly et al., 2001). TAMU will confine Coho salmon fingerlings in cages *in-situ* on contaminated Lake Union sediment. TAMU has prepared a QAPP describing the chemical, Microtox®, and DNA elements of the project under their direction (Donnelly et al., 2001).

The plan elements below address the *Hyalella* and *Chironomus* bioassays and the sediment field collection components under EAP direction. All of the data will be used to determine: 1) The most sensitive bioassay test for Lake Union/GWP sediments; 2) which tests correlate with alterations in DNA as measured by Texas A&M; 3) the nature and extent of sediment toxicity associated with Gas Works Park PAHs and metals.

Project Organization

KC Donnelly, Texas A&M Project Manager. Responsible for overall coordination of TAMU activities and budget.

Gary Barbee, Texas A&M Project Lead. Responsible for preparation of TAMU QAPP and final report. Conducts lab analysis of Ecology Microtox 100% porewater toxicity test (Adolphson, 2000). Reports chemical analyses conducted by TAMU contracted lab. Conducts evaluations of genotoxicity of GWP sediments using Coho salmon.

John Barich, USEPA Project Coordinator. Responsible for review of project documents and coordination of USEPA grant activities.

Richard Jack, Ecology Project Manager. Responsible for Ecology QAPP preparation, conducts sediment sampling, coordinates contract laboratory analysis of *Hyalella* and *Chironomus* bioassays, and prepares draft and final Ecology report describing results of chemical and biological analyses.

Brett Betts, TCP Project Coordinator. Responsible for review of QAPP and final reports for the project.

Schedule

Project Scoping – October 2001
Draft Ecology QAPP – November 2001
Final Ecology QAPP – February 2002
Field Sampling – March 2002
Laboratory Analysis Complete – June 2002
Draft Ecology Report – August 2002
Final Ecology Report – November 2002

Data Quality Objectives

Accuracy, Bias, and Precision

In an effort to minimize bias, Puget Sound Estuary Program (PSEP) (EPA, 1996) guidelines for collecting, preserving, transporting, and storing samples will be followed. Due to unknown factors influencing organism responses (overlying water quality, ammonia, salinity, and lab experience) quantitative determinations of accuracy for sediment bioassays are difficult (USEPA, 2000). Laboratory controls and reference sediments will assist in documenting accuracy of the bioassays as much as possible. Testing protocols will be followed closely to ensure that this study has no unique bias.

Representativeness

The objective of the study is to describe the nature and extent of sediment toxicity associated with sediments near Gas Works Park. To further this aim, composite samples will be collected from each location. Eleven test stations are proposed for sampling: two in the projected highest impact area, five in an anticipated transitional impact area, and four in a projected lowest impact area. Two reference locations in Lake Washington will also be sampled. The reference locations are near Webster Point. Previous King County (D. Houck, personal communication) sampling has confirmed that this location has a similar grain size and organic carbon content as sediments within Lake Union. Past bioassays have not revealed significant mortality at this location. The mean bioassay response from the reference stations will be used in determining study bioassay responses.

Completeness

A project goal of 100% useable data is targeted through planned fieldwork, careful sampling, packaging, and transport of samples. Spare samplers, containers, and other spare parts will be carried in the field as a preventative measure. In the event samples require re-testing, contract bioassay laboratories will hold any excess sediment at 4^0 C until holding times expire.

Comparability

Results from this study should be comparable with previous Lake Union, Lake Washington, and Salmon Bay studies. Sampling methods will be consistent with PSEP protocols (USEPA, 1996) and requirements of the Sediment Management Standards (Ecology, 1995a, b). Sampling, quality assurance, and analytical methods are also consistent with other sediment studies in the Lake Union environs. This study will record the locations of sampling stations with differentially corrected GPS to allow comparison with other sampling efforts.

The *Hyalella* and Microtox® tests are routinely employed in freshwater sediment studies throughout the state (Cubbage et al., 1997). *Chironomus* bioassay tests have historically been used in NPDES permitting but are now also commonly employed at Ecology's contaminated freshwater sediment sites (Johnson, 2000). Historically, the *Hyalella* and *Chironomus* tests have been of a shorter, 10-day duration. This study proposes to use a longer term testing period for these bioassays (28 and 20 days respectively), in an attempt to incorporate chronic effects. The chosen term lengths of these tests are accepted as industry standards (ASTM, 1996; USEPA, 2000). Both tests measure survival and growth endpoints.

EAP will review the TAMU QAPP to ensure that the chemical analyses and Microtox® data are accurate, representative, complete, and comparable with other EAP data.

Study Design

Samples will be collected from eleven locations in a radial pattern along anticipated contaminant gradients adjacent to GWP (Figure 1). Sampling stations were chosen based on previous Ecology, EPA (1985), and King County (Tomlinson et al., 1977 and Hansen, 1993) data. Two stations are located in areas with high contaminant concentrations. Five stations are located in areas with intermediate PAH concentrations. These seven stations will be used for comparisons with TAMU DNA mutation data. The last four test stations are presumed to be in areas with lower contaminant concentrations. They will close data gaps in the definition of nature and extent in the vicinity of Gas Works Park.

Two reference sites in Lake Washington adjacent to Webster Point will also be sampled. Samples near Webster Point have historically had low-level detections of organics (Grothkopp, 1997; NOAA, 1994); however, this location is not near any known sources of sediment or groundwater contamination. The Webster Point site is most appropriate as a reference because other upstream locations in Lake Washington have sand substrates. Webster Point has a more organic substrate, which more closely matches the grain size and organic carbon content of Lake Union sediments. Many other possible reference locations in Lake Union have other contaminant sources and are thus not appropriate as reference sites.

Field Procedures

Samples from all sites will be collected from an Ecology boat or from a USEPA vessel using a 0.1 m^2 stainless steel van Veen grab sampler. Sampling sites will be located using differentially corrected GPS and upland landmarks. Target coordinates for each station will be generated prior to the field collection. A field log will be maintained during sampling. See Appendix B for a sample log book page.

Grabs will be considered acceptable if the sampler is not overfull, overlying water is present and not significantly turbid, the sediment/water interface is relatively flat, and at least 11 cm of sediment depth is present.

Hyalella and *Chironomus* bioassay and analytical chemistry sediment samples will be composited from three individual grabs per station. The top 10-cm of sediment from each acceptable grab will be removed with stainless steel spoons, placed in a stainless steel mixing bowl, and homogenized. Materials touching the sides of the van Veen sampler will not be used. For the Microtox® bioassay, relatively undisturbed sediment will be collected, and not homogenized. Sample containers for Microtox® testing will be filled completely to minimize alterations in porewater chemistry.

Homogenized *Hyalella* and *Chironomus* bioassay sediments and analytical chemistry sediment samples will be placed in laboratory clean glass jars with Teflon lid liners. Container sizes, preservation, and holding times are shown in Table 2.

Prior to sampling, stainless steel sampling implements will be cleaned by sequentially:
1) Washing in Liquinox detergent and hot tap water;
2) Rinsing with hot tap water;
3) Rinsing with 10% nitric acid;
4) Rinsing with deionized water;
5) Rinsing with pesticide grade methanol;
6) Air-drying.

After drying, equipment will be wrapped in aluminum foil until used in the field. Sampling spoons and bowls will be dedicated to each station to avoid field decontamination procedures.

Only sediments not in contact with the van Veen sampler will be removed for homogenization and analysis. All samples will be cooled to 4^0 C immediately after collection and transported under chain-of-custody protocols.

Laboratory Practices

The proposed analytical methods for EAP analyzed materials are shown in Table 3. A contract laboratory will conduct both the 28-day *Hyalella* and the 20-day *Chironomus* testing. Both tests are modifications of other established, standardized tests (ASTM, 1996; USEPA, 2000). A Department of Ecology accredited laboratory, with recent and successful experience with these tests, will be contracted to conduct them. Estimated laboratory costs are provided in Table 4.

Quality Control Procedures

Field Measures

Decks and sampling areas will be washed off between sampling locations to prevent cross-contamination. Sampling will progress from the anticipated zones of lower contamination towards the more highly contaminated zones. Disposable nitrile® gloves will be used for each sampling location.

In general, excess sediment will be drummed for appropriate disposal. The van Veen grab will be field decontaminated after highly contaminated stations with Liquinox detergent and methanol. At stations without oily wastes which could cause a sheen, the van Veen will be decontaminated by brushing with on-site lake water.

Field variability within a sampling zone will be evaluated through comparison of the multiple stations within the zone. To qualitatively evaluate analytical precision, one split will be collected. Splits or duplicates will not be performed on bioassay samples.

Laboratory Measures

Two reference sediments will be collected from Lake Washington near Webster Point. Reference sediments will be roughly field matched to the grain sizes of test sediments if possible. The reference site has no known sources of sediment or groundwater contamination.

Holding times for bioassays are 14 days. The contract lab will archive any extra sediment to allow for the reinitiation of tests should early test failure occur (e.g. due to elevated salinity or ammonia).

For bioassays, acceptable quality controls are defined in the Table 5. These include measurements of hardness, dissolved oxygen, the use of a reference toxicant and other parameters. Table 5 presents the minimum monitoring frequencies and control limits for *Hyalella* and *Chironomus* bioassays. For physical parameters, acceptable quality control parameters are provided in Table 6.

Data Reduction and Management

Station field data will be recorded in field notebooks (Appendix B). Chemistry and Station identification data will be entered into Ecology's Environmental Information System (EIM). EAP is requesting that TAMU provide the chemical and Microtox data in a suitable electronic format to facilitate this entry. *Hyallela* and *Chrionomus* data should also be provided by the contract laboratory electronically. Both chemistry and bioassay data will be entered into SEDQUAL prior to completion of the final report.

Data Review and Validation

The laboratory's reporting of the bioassay results shall include the USEPA requirements listed in Appendix A. Their statistical analysis of the data will include comparisons to both the laboratory negative control and the reference sediments, using a t-test at a significance level of 0.05. All assumptions regarding the suitability of these tests will be confirmed by the project lead prior to use.

Upon receipt of the bioassay data, the project lead will review the results for completeness, reasonableness, and usability. The bioassay data will be reviewed to assure that the methods and test conditions were followed and that the results on negative controls and reference toxicants were acceptable. The project lead will also perform a second review of the chemical data provided by TAMU and their case narratives to assure that quality control procedures meet frequency requirements and control limits. After data review is completed, and following any necessary corrective actions, the complete biological and chemical data package will be forwarded to the client.

The project lead will provide a draft report of the study results to the client in August 2001. At a minimum, the final report will include the following:

- A study area map showing the sampling sites
- Latitude and Longitude and other information describing the sampling sites
- Descriptions of field and laboratory methods
- A data quality synopsis and discussion of the significance of any analytical problems
- Summary tables of biological and chemical data
- An evaluation of significant findings
- Recommendations for follow-up work as deemed necessary

A final report will be prepared following receipt of comments from the client and internal EAP review. The final report goal is November 2002. In addition, data will be entered into EIM and SEDQUAL.

Data Quality Assessment

Data will be reviewed prior to completion of the final report to ensure the DQOs have been met. The final report will note any QA/QC discrepancies, which may influence their use. A recommendation will be provided regarding the most sensitive bioassay test for Lake Union/GWP sediments. Depending on the timing and results from Texas A&M, DNA tests which correlate with established sediment toxicity measures will be presented. The strengths and weaknesses of any relationships will be discussed. The nature and extent of sediment toxicity associated with Gas Works Park PAHs and metals will be summarized. Gaps in this definition will be identified and recommendations for further testing will be made as warranted.

Table 1. Target Analyte List for Gas Works Park Sediment Analysis; Collected by EAP and Analyzed by TAMU

Metals	Organics	Aggregate Measures
Arsenic	Acenaphthene	Total Organic Carbon
Cadmium	Acenaphthylene	Grain Size
Chromium	Fluorene	
Copper	Benzo(a)anthracene	
Lead	Chrysene	
Mercury	Pyrene	
Silver	Benzo(a)pyrene	
Zinc	Dibenzo(a,h)anthracene	
	Indeno(1,2,3,-c,d)pyrene	
	Benzo(g,h,i)perylene	
	2-methylnaphthalene	
	Dibenzofuran	
	1,2-dichlorobenzene	
	1,4-dichlorobenzene	
	1,2,4-trichlorobenzene	
	Hexachlorobenzene	
	Dimethyl phthalate	
	Diethyl phthalate	
	Di-n-Butyl phthalate	
	Butyl benzyl phthalate	
	Bis (2-ethylhexyl)phthalate	
	Di-n-octyl phthalate	
	Hexachlorobutadiene	
	n-Nitrosodiphenylamine	
	Phenol	
	2-methylphenol	
	4-methylphenol	
	Pentachlorophenol	
	Benzyl Alcohol	
	Benzoic Acid	
	Total PCBs (Aroclors)	
	Tributyltin	

Table 2. Sample Containers, Preservation, and Holding Times for Lake Union Sediments

Analysis	Container	Preservation	Holding Time
Bioassay:			
Chironomus	1-gallon glass, PFTE lid	4 C, in dark	14 days
Hyalella	1-gallon glass, PFTE lid	4 C, in dark	14 days
Microtox®	1-L glass, PFTE lid	4 C, in dark	14 days
Chemistry:			
Organics/semivolatiles	8 oz. Glass, PFTE lid	4 C, in dark	14 days
Metals	8 oz. Glass, PFTE lid	4 C, in dark	6 months
TOC	8 oz. Glass, PFTE lid	4 C, in dark	14 days
Grain Size	Included with above	N/A	6 months
Percent Solids	Included with above	N/A	6 months

Table 3. Analytical Methods, Required Reporting Limiting, and Laboratories for Lake Union Sediments

Analysis	Reporting Limit	Method	Laboratory
Bioassay:			
Chironomus 20-day	N/A	Method 100.5 (EPA, 2000)	Contractor
Hyalella 28-day	N/A	Method 100.4 (EPA, 2000)	Contractor
Chemistry:			
TOC	0.1%	Combustion, (EPA, 1996)	Manchester
Grain Size (gravel, sand, silt, clay fractions)	0.1% per fraction	Sieve & Pipet (EPA, 1996)	Contractor
Percent Solids	0.1%	Gravimetric, (EPA, 1996)	Manchester

Table 4. Cost Estimate for Lake Union Sediment Analysis

Analysis	Cost per Sample	Sample Number	Subtotal
Hyalella 28-day	$1150	13	$14950
Chironomus 20-day	$960	13	$12480
TOC	$33	13	$429
Grain Size	$100	13	$1300
% Solids	$10	13	$130
		Total	$29,289

Table 5. Allowable Test Conditions for Lake Union Sediment Bioassays

Test Species	**Water Quality Monitoring Frequency**		**Control Limits**			**Laboratory Controls**		**Test Acceptability**
	Hardness, alkalinity, conductivity, salinity pH, NH_3	Temperature, Dissolved O_2	Temp.	D.O. (% saturated)	Negative Control	Reference Toxicant	Reference Sediment	
Hyalella azteca	Day 1, 14, 28	Daily	23 C ±1	>40%	Yes, clean sand or reference	KOH	Yes	Mean control survival ≥80%; mean weight of surviving controls ≥0.1 mg
Chironomus tentans	Day 1, 10, 20	Daily	23 C ±1	>40%	Yes, clean sand or reference	KOH	Yes	Mean control survival ≥70%; mean weight of surviving controls ≥0.6 mg

Table 6. Analysis Quality Control Requirements for Physical Parameters of Lake Union Sediment

	TOC	**Grain Size**	**% Solids**
Laboratory Duplicates	≤20% RPD	≤20% RPD	≤20% RPD*

* Relative percent difference

References

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Appendix A

Reporting Requirements for Sediment Bioassays (USEPA, 2000)

1) The record of the results of an acceptable sediment test should include the following information either directly or by reference:
   A) Name of the test and investigators, name and location of the laboratory, and test start and end dates.
   B) Source of control and test sediments and methods for: collection, handling, shipping, storage, and disposal.
   C) Source of test material, including lot numbers as applicable, composition of major ingredients and any known impurities, known physical and chemical properties, and name and concentrations of any solvents used.
   D) Source and characteristics of overlying water, description of any pretreatment, and results of any demonstrations of organism viability in the water.
   E) Source, history, and age of test organisms; source, history, and age of brood stock; culture procedures; dates of collection if applicable; scientific name; name of individual who identified the organisms and taxonomic key used; age or life stage; mean and ranges of weight or length; any unusual appearance, diseases, or treatments used; and holding procedures.
   F) Source and composition of food, concentrations of test material and other contaminants, procedures used to prepare food, feeding methods, frequency, and duration.
   G) Description of the experimental design and test chambers, depth and volumes of sediment and overlying water in the chambers, lighting, number of test organisms per treatment, date and time of test initiation and termination, temperature measurements, dissolved oxygen concentration (μg/L) and any aeration used before and during the tests.
   H) Methods used for physical and chemical characterization of sediment.
   I) Definitions of the effects used to calculate LC_{50} and/or EC_{50}, biological endpoints for tests, and a summary of general observations of other effects.
   J) A table of the biological data for each test chamber for each treatment, including control(s), in sufficient detail to allow independent statistical analysis.
   K) Methods used for statistical analysis.
   L) Summary of general observations on other effects or symptoms.
   M) A description about any unusual test conditions, any procedural deviation, and any other relevant information.

2) Published reports should contain enough information to clearly identify the methodology used and the quality of the results.

Appendix B, Lake Union Sediment Sampling Field Data

Sample ID	General Location	General Sediment Type	Latitude	Longitude	Collection Start Time	Collection Stop Time	Grab 1 overlying water depth	Grab 2 overlying water depth	Grab 3 overlying water depth