

Reassessment of Toxicity of Lake Roosevelt Sediments

Quality Assurance Project Plan

by
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Washington State Department of Ecology
Environmental Assessment Program
Olympia, WA 98504-7710

303(d) listings addressed in this study:

Franklin D. Roosevelt Lake (WA-CR-1060) – Sediment Bioassay Toxicity

Approvals:

_____ Dave Knight, ERO Water Quality Program	_____ Date
_____ Carl Nuechterlein, Section Manager, ERO Water Quality Program	_____ Date
_____ Dave Serdar, Project Manager, Watershed Ecology Section	_____ Date
_____ Brandee Era, Principal Investigator, Watershed Ecology Section	_____ Date
_____ Dale Norton, Unit Supervisor, Contaminant Studies Unit	_____ Date
_____ Will Kendra, Section Manager, Watershed Ecology Section	_____ Date
_____ Stuart Magoon, Director, Manchester Environmental Laboratory	_____ Date
_____ Cliff Kirchmer, Ecology Quality Assurance Officer	_____ Date

Background and Problem Statement

Studies published by the Washington State Department of Ecology (Ecology) and the United States Geological Survey (USGS) revealed sediment toxicity in Franklin D. Roosevelt Lake and the upstream reach of the Columbia River (Johnson, 1991; Bortelson et al., 1994). Results provide the basis for nine separate entries on the Clean Water Act 303(d) list for 1998. Although these sites are listed solely from sediment bioassay results, data from these studies strongly suggest that the bioassay toxicity was likely due to metals contamination of the bed sediments. The Cominco Ltd. lead-zinc smelter in Trail, B.C., located approximately ten river miles above the international border, is the primary source of metals contamination (Johnson et al., 1988; Bortelson et. al, 1994; Serdar, et al., 1994). Mining activity in the watershed may also be a significant source, especially in lower Lake Roosevelt. Metals found at high concentrations in Lake Roosevelt and the upper Columbia River include zinc, lead, copper, arsenic, cadmium, and mercury.

Project Description

Ecology's Eastern Regional Office Water Quality Program (WQ/ERO) has requested a reassessment of sediment toxicity of sediments from Lake Roosevelt and the upstream reach of the Columbia River since existing data are a decade old (Johnson, 1991; Bortelson et al., 1994). The Environmental Assessment Program (EAP) will sample sediments at the nine sites previously found to have sediment toxicity, plus a reference site. Samples will be analyzed for sediment bioassays and metals in order to determine whether sediments from these sites remain toxic (see below). Based on the findings, EAP will recommend either retaining or removing these sites from the 303(d) list.

Bioassays

Chironomus tentans 20-day Survival and Growth (Chronic)

Hyalella azteca 10-day Survival (Chronic)

Microtox® 100% Porewater (Acute)

Chemistry

Total Metals: Arsenic, Cadmium, Copper, Lead, Mercury and Zinc

Percent Solids

Grain Size

Total Organic Carbon

Project Organization

Project Manager - Dave Serdar (360)407-6772

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WQ ERO Section Manager - Carl Nuechterlein (509)456-6198

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Manchester Laboratory Director - Stuart Magoon (360)871-8801

Manchester Laboratory Bioassay Contracts - Pam Covey (360)871-8827

Quality Assurance Officer - Cliff Kirchmer (360)4076455

Bioassay Laboratories - Gerald Irissari, Northwestern Aquatic Sciences (541)265-7225
Jim Laughlin, Parametrix (425)822-8880

Schedule

May 7-10, 2001	Sediment Samples Collected and Submitted for Analysis
July 1, 2001	Laboratory Analyses Completed and Results Reported to Project Manager
July 2001	Data Entered into EIM
September 2001	Draft Report to Client
October 2001	Final Report

Data Quality Objectives

Accuracy, Bias, and Precision

PSEP procedures (EPA, 1996) for collection, preservation, transportation, and storage of sediment samples will be followed in an effort to limit sources of bias.

Due to the unknown variables that affect organism response, overlying water quality, and the experience of laboratory personnel, quantitative determination of accuracy in sediment testing of aquatic organisms is difficult (EPA, 2000). Since there is no acceptable reference material suitable for determining the accuracy of sediment tests, the accuracy of these test methods has not been determined (EPA, 2000a). It therefore becomes important that the testing protocols are followed closely.

The data quality objective for field split samples in this study is 25% relative standard deviation (RSD). The RSD for field splits will give an indication of overall data precision.

Representativeness

The objective here is to obtain samples which are representative of bottom sediments at the locations targeted. Composite samples will be collected in an effort to obtain data representative of each sampling site.

Completeness

The objective of obtaining 100% useable data will be maximized by careful planning of fieldwork, packaging, and transport of samples. The contract laboratories will hold excess samples for 60 days at 4° C, in the event that the samples need to be re-analyzed. Manchester Laboratory will save excess sample for 60 days (at 4° C) from the time the data is sent to the project lead to give time for its review.

Comparability

Results obtained from this study should be comparable to other EAP studies and those conducted in Lake Roosevelt. Sampling methods will be consistent with PSEP protocols (EPA, 1996) and requirements of the Sediment Management Standards (Ecology, 1995a,b). The sampling, quality assurance, and analytical methods selected for the chemical analyses are consistent with those used in other sediment sampling efforts on Franklin D. Roosevelt Lake and the upper Columbia River reach (Bortelson et al., 1994; Johnson, 1991). Station positions will be recorded using global positioning system (GPS) to allow comparison with previous sampling locations.

The *Hyalella* 10-day acute bioassay is routinely employed in freshwater sediment studies throughout the state (Cubbage et al., 1997). The Microtox® porewater and *Chironomus* bioassays are newly developed but are now commonly used at Ecology's contaminated sediment sites (e.g., Johnson, 2000; ThermoRetec Consulting Corp., 2000). The 20-day *Chironomus* test incorporates growth and survival endpoints and increases the sensitivity of the test.

Study Design

Samples will be collected from nine stations in Lake Roosevelt to assess current sediment toxicity. The proposed stations correspond to the nine locations currently listed on the section 303(d) list of impaired waterbodies. Sampling locations are shown in Table 1 and Figure 1. The reference site will be located in Lower Arrow Lake, Canada.

Field Procedures

Samples from all the sites will be collected from Ecology's 20 ft skiff using a 0.1 m² stainless steel van Veen grab. Sampling sites will be located and positions recorded using GPS and landmarks. 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 desired depth penetration has been achieved. A field log will be maintained during sampling. All samples will be composites of the top 10 cm layer to evaluate the biologically active layer (Ecology, 1995b; EPA, 2000). After siphoning off overlying water, the top 10 cm of sediment from each of three-to-five individual grabs per sampling site will be removed with stainless steel scoops, placed in a stainless steel bowl, and homogenized by stirring together the contents of the replicate grabs. Material touching the sidewalls of the grab will not be taken. The samples for the Microtox test will be taken with minimum disturbance of the sediment, not homogenized, and the sample containers filled completely (no headspace) to minimize changes in pore water chemistry.

The homogenized sediment will be placed in glass jars with Teflon lid liners and cleaned to EPA QA/QC specifications (EPA, 1990). Sample containers, preservation, and holding times are shown in Table 2. Excess samples will be retained from each composite and stored frozen in the event that additional analysis is required.

Stainless steel implements used to collect and manipulate the sediments will be cleaned by washing with Liquinox detergent and followed by sequential rinses with tap water, 10% nitric acid, and deionized water. The equipment will then be air-dried and wrapped in aluminum foil. Between-sample cleaning of the van Veen grab will consist of thorough brushing with on-site water.

Sediment samples will be placed on ice immediately after collection and transported to Manchester Laboratory within one-to-two days. Chain-of-custody will be maintained. Manchester will ship the bioassay samples to the contract laboratories.

Back-up sampling equipment, sample containers, positioning instruments, and spare parts will be carried during field sampling as preventative maintenance.

Laboratory Procedures

Table 3 shows the analytical methods that will be used, the reporting limits for the chemical analyses, and laboratories selected to conduct the work. Northwestern Aquatic Sciences will conduct the *Hyalella* 10-day tests and the *Chironomus* 20-day test. Parametrix will conduct the Microtox® test. These bioassays were recently developed and no laboratories have been accredited for these specific tests. Parametrix is accredited for the 1995 PSEP Microtox test. Northwestern Aquatic Sciences is accredited for shorter-term ASTM methods for both *Hyalella* and *Chironomus*. These laboratories have had recent and successful experience in conducting the tests being requested of them.

In the Microtox® test, the light emitted by the bioluminescent marine bacterium *Vibrio fischeri* on exposure to test sediments is compared to a control or reference sample. The 100% porewater test is an Ecology modification (Adolphson, 2000) of Puget Sound Estuarine Protocols (PSEP) that use organic or aqueous extracts (see Appendix A).

Hyalella azteca is an amphipod. The test measures survival after a 10-day exposure to test sediment (ASTM E-1706 and EPA method 100.1).

Growth and survival of the midge, *Chironomus tentans*, are the end points of this 20-day chronic test. The method is a modification of a 50-to-65 day life-cycle test (Method 100.5; EPA 2000).

Table 4 shows the estimated laboratory costs for the project. The number of chemistry samples shown includes a laboratory duplicate.

Quality Control Procedures

Field Quality Control

Decks and sampling areas will be washed down between sampling sites to prevent cross-contamination of samples. Nitrile gloves will be worn when handling sediments. All implements used to manipulate the sediments will be clean (see Field Procedures section).

One sample will be split in the field to estimate sampling precision.

Lab Quality Control

The reference sediment for the bioassays will either be from Lower Arrow Lake Site 10 (a mainstream Columbia River reservoir upstream of Lake Roosevelt) or from Cedar Lake (located east of Northport on the Upper Columbia River reach). Both of these possible reference sites have no known source of metals or other contamination. If available, Manchester Laboratory may also analyze a certified reference standard sediment sample for metals.

Details of the QC procedures to be followed for the sediment bioassays are described in the methods referenced in this QAPP. Eight replicates each will be used for the *Hyalella* and *Chironomus* tests. The Microtox® test uses five replicates. Frequency of water quality monitoring, control limits, control samples, and test acceptability criteria for the bioassays are listed in Appendix B.

The holding time for metals is 6 months and 14 days for sediment bioassays at 4° C (EPA, 2000). The samples will be analyzed well before their holding times expire.

The QC procedures routinely followed by Manchester Laboratory for the chemical analyses requested will be satisfactory for purposes of this project. QC samples for the metals analysis will include a laboratory duplicate, method blank, one matrix spike, one matrix spike duplicate, and a laboratory control sample (LCS). All QC samples will be analyzed from the same batch as the test samples. The project lead will identify the sample to be used for the duplicate and matrix spikes. QC samples for TOC and percent solids will include a laboratory duplicate and a LCS (TOC only). A laboratory triplicate analysis will be conducted for grain size.

Table 5 shows the quality control analysis requirements for this project. Matrix spikes may provide an indication of bias due to interference from the sample matrix. The laboratory control sample (metals and TOC) recoveries will provide an estimate of accuracy for the entire analytical procedure. Overall precision of the chemical data will be estimated from the results of duplicate analyses and matrix spike/matrix spike duplicates.

Data Reduction and Management Procedures

Station data and field data will be written in field notebooks and will then be entered into the Environmental Information System (EIM) within two weeks of being collected. After laboratory data is reviewed, it will also be entered into EIM. All data will be entered in EIM before the final report is complete.

Data Review and Validation

The laboratories reporting of the bioassay results must include the EPA (2000) requirements listed in Appendix C. Their statistical analysis of the data will include comparison to both the laboratory negative control and the reference sediment (Site 10), using a t-test at a significance level of 0.05.

Manchester's SOP for reduction, review, and reporting of the chemical data will meet the needs of this project. Each laboratory unit assembles data packages consisting of raw data from the analyses of the samples, copies of the pertinent logbook sheets, QA/QC data, and final reports of data entered into LIMS. These data packages are subjected to a data verification and quality assurance review by another analyst familiar with the procedure. Reviewers use *USEPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review*, February 1994 and *USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review*, October 1999.

On receipt of the bioassay and chemical 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 results on negative controls and reference toxicants were acceptable. The chemical data and case narratives will be reviewed to assure that quality control procedures meet frequency requirements and control limits. After data review is completed and following any corrective actions required, the complete biological and chemical data will be forwarded to the client.

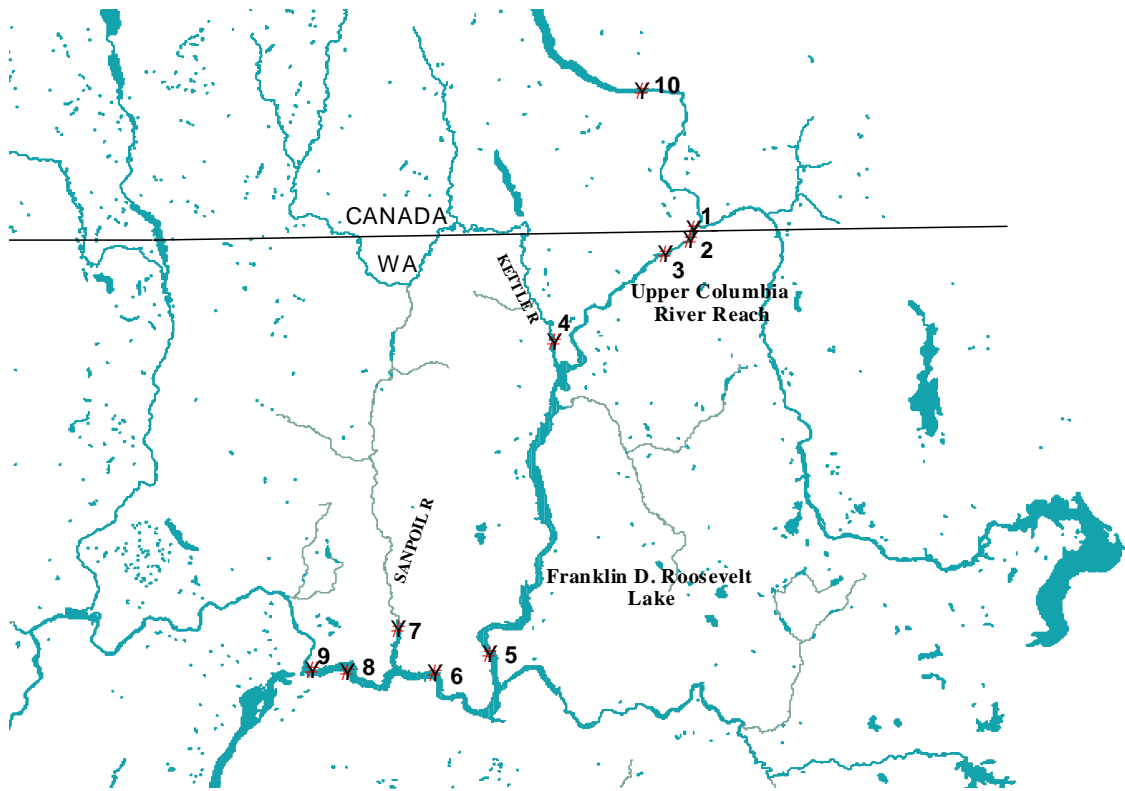
The project lead will provide a draft report of the study results to the clients in September 2001. At a minimum the final report will contain the following:

- A map of the study area showing sampling sites
- Latitude/longitude and other location information for each sampling site
- Descriptions of field and laboratory methods
- A discussion of data quality and the significance of any problems encountered in the analyses
- Summary tables of the biological and chemical data
- An evaluation of significant findings
- Recommendations for follow-up work

A final report will be prepared after receiving review comments from WQ - ERO and internal comments from EAP. The goal is to have the revised, final report completed by September 2001. The data will be entered into Ecology's Environmental Information Management (EIM) system and made available electronically for entry into SEDQUAL.

Data Quality Assessment

The data will be reviewed to make sure the data satisfactorily meets the data quality objectives (DQOs) and will be noted in the final report.



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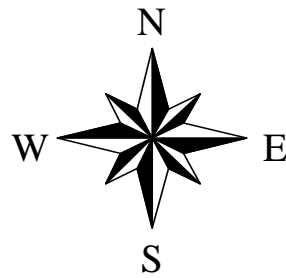


Figure 1. Sampling Sites for the Lake Roosevelt Sediment Study

Table 1. Sediment Sampling Locations for Lake Roosevelt

Site No.	Old Site No.	Site Name	River Mile	Tributary Mile	Latitude	Longitude
1	8	Boundary LB	745		48 59 56	117 37 51
2	10	Auxiliary Gage LB	743		48 58 24	117 38 48
3	11	Goodeve Creek RB	738		48 56 23	117 43 55
4	25	Kettle River RB1	707	4.3	48 44 04	118 06 57
5		Castle Rock	644.8		47 58 40*	118 21 55*
6	61	Whitestone Creek MS	621		47 55 56	118 32 54
7	62	Sanpoil River MS	616	7.2	48 02 34	118 39 54
8		Swawilla Basin	604.9		47 56 35*	118 50 30*
9	71	Grand Coulee Dam RB	596		47 56 45	118 57 30
10	1	Lower Arrow Lake LB1 (reference site)	787		49 20 20	117 47 09

* Positions from Johnson, et. al., 1988; others from Bortelson, et. al., 1994.

Table 2. Sample Containers, Preservation, and Holding Times

Analysis	Container	Preservation	Holding Time
Bioassays			
<i>Chironomus</i>	1-liter glass; TFE-lined lid	4 deg C in the dark	14 days
<i>Hyalella</i>	1-liter glass; TFE-lined lid	4 deg C in the dark	14 days
Microtox	1-liter glass; TFE-lined lid	4 deg C in the dark	14 days
Chemistry			
Metals	8 oz glass; TFE-lined lid	4 deg C in the dark	6 months
TOC	4 oz glass; TFE-lined lid	4 deg C in the dark	28 days (1 year frozen)
% Solids	8 oz glass; TFE-lined lid	4 deg C in the dark	7 days
Grain size	8 oz glass; TFE-lined lid	4 deg C in the dark	6 months

Table 3. Analytical Methods, Reporting Limits, and Laboratories

Analysis	Reporting Limit	Method	Laboratory
Bioassays			
<i>Chironomus</i> 20-day	n/a	Method 100.5 (EPA, 2000)	NW Aquatic Sciences
<i>Hyalella</i> 10-day	n/a	ASTM E-1706 and Method 100 (EPA, 2000)	NW Aquatic Sciences
Microtox pore water	n/a	Ecology Protocol	Parametrix
Chemistry			
Arsenic	4 mg/Kg, dry	ICP/AES – EPA3050/6010B	Manchester
Cadmium	0.5 mg/Kg, dry	ICP/AES – EPA3050/6010B	"
Copper	1 mg/Kg, dry	ICP/AES – EPA3050/6010B	"
Lead	3 mg/Kg, dry	ICP/AES – EPA3050/6010B	"
Mercury	0.003 mg/Kg, dry	CVAA - EPA245.5	"
Zinc	0.5 mg/Kg, dry	ICP/AES – EPA3050/6010B	"
TOC	0.1%	Combustion/CO2 - EPA (1996)	"
% Solids	0.1%	Gravimetric - EPA (1996)	"
Grain Size*	0.1%	Sieve & Pipet - EPA (1996)	"

*Gravel, sand, silt and clay fractions

Table 4. Cost Estimate for Analyzing Franklin D. Roosevelt Lake Sediment Samples

Analysis	Number of Samples	Matrix Spikes	Total Analyses	Cost/Analysis	Cost Subtotals
Bioassays					
<i>Chironomus</i> 20-day	10	n/a	10	920	9200
<i>Hyalella</i> 10-day	10	n/a	10	560	5600
Microtox	10	n/a	10	450	4500
					19300
			25% Manchester surcharge		4825
					<u>\$ 24,125.00</u>
Chemistry					
Metals	12	2	14	118	1652
TOC	12	0	12	33	396
% Solids	12	0	12	10	120
Grain Size	12	0	12	100	1200
					<u>\$ 3,368.00</u>
			Total cost		\$ 27,493.00

Table 5. Quality Control Analysis Requirements

	TOC	Grain Size	Metals	Bioassays
Matrix spikes	NA	NA	75-125% recovery	NA
Matrix spike duplicates	NA	NA	≤ 25% RPD	NA
Laboratory Control Samples	NA	NA	Within method control limits	NA
Laboratory duplicates	≤ 20% RPD	≤ 20% RPD	≤ 20% RPD	NA
Standard reference material	NA	NA	Accepted range of certified values	NA

NA=Not Applicable

RPD=Relative Percent Difference

References

- Adolphson, P. 2000 (Draft Final). *Microtox® 100 Percent Sediment Porewater Toxicity Assessment*. Washington State Department of Ecology, Olympia, Washington.
- Bortleson, G., S. E. Cox, M. D. Munn, R. J. Schumaker, E. K. Block, L. R. Lucy, S. B. Cornelius, 1994. *Sediment-Quality Assessment of Franklin D. Roosevelt Lake and the Upstream Reach of the Columbia River, Washington, 1992*. U.S. Geological Survey. Open File Report 94-315.
- Cabbage, J., D. Batts, and S. Breidenbach. 1997. *Creation and Analysis of Freshwater Sediment Quality Values in Washington State*. Washington State Department of Ecology, Olympia, Washington. Publication Number 97-323a.
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- Johnson, A. 2000. *Quality Assurance Project Plan for Toxicity Testing of Spokane River Sediments*. Washington State Department of Ecology, Olympia, Washington.

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Appendices

Appendix A

Department of Ecology Microtox Porewater Protocol (Draft Final 8/15/00, Peter Adolphson)

Microtox® 100 Percent Sediment Porewater Toxicity Assessment

Background

Microtox is a rapid method of assessing toxicity in aqueous media by utilizing the bioluminescent properties of the marine bacteria *Vibrio fischeri*. The test method assumes that light emitted by the bacteria can be used as an accurate assessment of the overall biological condition of the bacteria exposed to chemical compounds and mixtures. Light emitted by the bacteria exposed to potentially toxic samples is compared to light emitted to unexposed bacterial controls. Differences in luminescence are therefore deemed an indication of relative toxicity.

EPA has recommended Microtox for TIE/TRE applications (EPA/600/2-88/070) as well as stormwater investigations. Successful applications also include NPDES compliance and sediment evaluations in freshwater, estuarine, and marine applications. Washington State PSEP (Puget Sound Estuarine Protocols) uses both an organic and an aqueous extraction protocol to assess sediment toxicity.

Recognizing that the goal of most sediment toxicity studies is to determine if ecologically/toxicologically significant differences exist between reference and investigative site sediments, four significant differences exist between the PSEP protocol and this revised protocol. 1) Extraction procedures are 100% pore water extraction rather than complex organic and aqueous extractions; 2) No serial dilutions are performed because LC50 calculations are not required to assess sediment toxicity between reference and site sediments; 3) No MOAS (Microtox Osmotic Adjusting Solution) is utilized; and 4) Statistical procedures utilize standard Analysis of Variance (ANOVA) or t-test procedures.

Microtox Test Procedure

Porewater Extraction and Adjustment

The general Microtox procedure involves centrifugation of 500 ml of both reference and test sediments at approximately 4500G in for 30 minutes resulting in approximately 50 ml of pore water. Approximately 25 mls of pore water are then pipetted into a clean glass container. The remaining porewater volume is set aside if needed for reducing salinity should the initial salinity adjustments steps outlined below result in the sample exceeding 22ppt.

The sample is then adjusted for salinity, dissolved oxygen and pH in the following order:

- 1) Salinity is adjusted to 20 ± 2 ppt using commercially available dry bulk marine aquarium reef salts (e.g. Forty Fathoms Reef®). **[Note: The salinity adjustment step is omitted for Marine and estuarine sediments whose porewater exceeds 20ppt salinity.]**
- 2) The dissolved oxygen (DO) is then adjusted by gentle aeration or agitation until it is between 50-100% saturation.
- 3) The pH of the salinity and DO adjusted reference and test sediment pore water should not differ from each other by more than 0.4 pH units. The pH is adjusted to 7.9-8.2 (if necessary) using a micropipette and a dilute solution (0.5 N) NaOH or HCl. Total volume of NaOH and/or HCl should be recorded. Final concentration [compared with 100% porewater extracted] can then be calculated using these data. Final dilution should not be reduced below 90% of the pore water extract. **[Note: The control solution is prepared by using deionized or distilled water and adjusting salinity, DO and pH as described above.]**

Preparation of Bacterial Suspension and Bioassay Test Setup

A vial of freeze-dried bacteria is rehydrated with 1.0 ml of Microtox® Reconstitution solution and allowed to equilibrate for 30-90 minutes in the 4-degree Microtox Analyzer well. ***[NOTE: Mixing of the reconstituted bacteria is essential. Mix the reconstituted solution with a 1 ml pipette a minimum of 20 times by pipetting. First pipette the solution from the bottom of the cuvette and deposit the pipetted solution on the surface of the liquid remaining in the cuvette. Then pipette 1 ml of solution from the bottom of the cuvette and slowly pipette the liquid into the bottom of the cuvette.]***

One (1.0) ml of control solution is then placed in each of 5 test cuvettes and placed into the 15-degree incubation chambers. This procedure is followed for the laboratory control solution, reference sediment porewater samples, and test sediment porewater samples for up to 4 test sediments/batch (5 pseudo-replicates per site).

In each of the test, reference, and control sample cuvettes, 10 uL of rehydrated bacteria suspension is added at 30 second intervals, immediately mixed using a 1ml pipette and allowed to incubate for 5 minutes. Used pipette tips are replaced with clean tips after each series of 5 pseudo-replicates (ref, control, and each test series ex: A1-A5). ***[NOTE: Extreme care must be used when pipetting these low volumes as slight residual amounts or presence of air bubbles in the pipette may cause variation due to error by as much as 100%.]***

Data Collection

At the initial (I_0) 5 minute mark, the first control vial is placed into the read chamber to “set” the instrument. At 30-second intervals, each cuvette (inclusive of A1) is placed into the read chamber for the initial reading (I_0). After 5 additional minutes, a second reading (I_5) is obtained following the above procedure. A 15-minute (I_{15}) is obtained in an additional 10 minutes.

Data Analysis

Statistical calculations are performed using a standard t-test by comparing reference with test site data. No gamma correction is required. Statistically significant differences with $\alpha = 0.05$ and the following relative differences are indications of test failure.

Control output should exceed 80 percent at the 5-minute reading and 65% at the 15-minute reading.

Appendix B

QC Requirements for Sediment Bioassay (Modified from Ecology, 1995b)

Table B1. Sediment Toxicity Test Conditions

Toxicity Test <i>Test Species</i>	Frequency of Water Quality Monitoring		Control Limits		Control Samples			Test Acceptability
	Hardness, alkalinity, conductivity, pH, ammonia	Temp., D.O.	Temp (°C)	Dissolved Oxygen (% sat.)	Negative Control	Positive Control	Reference Sediment	
Amphipod <i>Hyalella azteca</i>	Beginning and end of test	Daily	23±1	>40%	Clean sediment*	Reference toxicant Cadmium	Yes	Mean survival in control sediment ≥80 percent. Mean weight of surviving controls ≥ 0.1 mg
Midge <i>Chironomus tentans</i>	Beginning and end of test	Daily	23±1	>40%	Clean sediment*	Reference toxicant Cadmium	Yes	Mean control survival ≥ 70% and minimum weight of survivors 0.6 mg
Microtox® (100% pore water) <i>Vibrio fischeri</i>	NA	NA	15	NA	Control solution*	Reference toxicant Cadmium	Yes	Control output > 80% @ 5 minutes and >65% @ 15 minutes

* Negative control sediments provided by the laboratory

Appendix C

Reporting Requirements for Sediment Bioassays (From EPA, 2000)

16.4 Reporting

16.4.1

The record of the results of an acceptable sediment test should include the following information either directly or by referencing available documents:

16.4.1.1

Name of test and investigator(s), name and location of laboratory, and dates of start and end of test.

16.4.1.2

Source of control or test sediment, and method for collection, handling, shipping, storage and disposal of sediment.

16.4.1.3

Source of test material, lot number if applicable, composition (identities and concentrations of major ingredients and impurities if known), known chemical and physical properties, and the identity and concentration(s) of any solvent used.

16.4.1.4

Source and characteristics of overlying water, description of any pretreatment, and results of any demonstration of the ability of an organism to survive or grow in the water.

16.4.1.5

Source, history, and age of test organisms; source, history, and age of brood stock, culture procedures; and source and date of collection of the test organisms, scientific name, name of person who identified the organisms and the taxonomic key used, age or life stage, means and ranges of weight or length, observed diseases or unusual appearance, treatments used, and holding procedures.

16.4.1.6

Source and composition of food; concentrations of test material and other contaminants; procedure used to prepare food; and feeding methods, frequency and ration.

16.4.1.7

Description of the experimental design and test chambers, the depth and volume of sediment and overlying water in the chambers, lighting, number of test chambers and number of test organisms/treatment, date and time test starts and ends, temperature measurements, dissolved oxygen concentration ($\mu\text{g/L}$) and any aeration used before starting a test and during the conduct of a test.

16.4.1.8

Methods used for physical and chemical characterization of sediment.

16.4.1.9

Definition(s) of the effects used to calculate LC50 or EC50s, biological endpoints for tests, and a summary of general observations of other effects.

16.4.1.10

A table of the biological data for each test chamber for each treatment, including the control(s), in sufficient detail to allow independent statistical analysis.

16.4.1.11

Methods used for statistical analyses of data.

16.4.1.12

Summary of general observations on other effects or symptoms.

16.4.1.13

Anything unusual about the test, any deviation from these procedures, and any other relevant information.

16.4.2

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