Verification of 303(d)-listed Sites in Northwest, Central, and Eastern Regions of Washington State

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Verification of 303(d)-listed Sites in Northwest, Central, and Eastern Regions of Washington State

by
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July 2004

Waterbody Numbers:
47122G4H1 (Shilshole Bay/ Central Puget Sound)
TS53NN (Springbrook/ Mill Creek)
KN36FW (Icicle Creek)
HM20EV (Wenatchee River)
QZ45UE (Spokane River)

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Table of Contents

List of Figures and Tables

Page

Figures

Tables

Abstract

Five waterbodies located among three of the Washington State Department of Ecology's management regions, Northwest, Central, and Eastern, were re-assessed for violations of water quality standards. Due to water quality violations, these waterbodies were previously listed or were proposed for listing on the current 2002/2004 Section 303(d) List of the federal Clean Water Act.

The following recommendations for the 303(d) List were made, based on the findings of this re-assessment study:

- Northwest Region: List Shilshole Bay for total PCBs in fish tissue (a new listing), but de-list it for dieldrin in fish tissue. Continue listing Mill Creek based on sediment bioassay toxicity.

- Central Region: List the Wenatchee River for total PCBs and 4,4'-DDE in fish tissue, but de-list it for alpha-BHC, 4,4'-DDD, and 4,4'-DDT in fish tissue. List Icicle Creek, a major tributary to the Wenatchee River, for total PCBs in fish tissue.

  Buffalo Lake, located in the Central Region, was selected as the reference site for the Spokane River (Eastern Region) sediment toxicity listing. Buffalo Lake sediments were found to have toxicity, but due to insufficient data, the lake is recommended for Category 2 (*Waters of Concern*).

- Eastern Region: Continue listing the Spokane River for sediment bioassay toxicity.

Acknowledgements

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  - Peter Adolphson and Brett Betts, Toxic Cleanup Program Sediment Management Unit, for their help in interpreting the sediment bioassay data.
  - Joan LeTourneau for formatting and editing the final report.

Background

The Washington State Department of Ecology's (Ecology) Water Quality Program requested that five waterbodies be re-assessed for violations of water quality standards. The locations of these areas are shown in Figure 1. These waterbodies were previously listed or are proposed for listing on the federal Clean Water Act, Section 303(d) List because of water quality violations. The Water Quality Program is currently in the process of preparing the 2002/2004 Section 303(d) List and wanted more information in order to determine the appropriateness of each listing. Some of the current listings are based on data that are more than 20 years old or data that are of questionable accuracy.

A summary of the current listings by region is shown in Table 1. Detailed decision matrices for each of the 303(d) waterbody listings are included in Appendix A.

Table 1. Individual 303(d) Listings Addressed by this Verification Study

Waterbody Name	Matrix	303(d)-listed Parameter	Current Waterbody ID Number	Old Waterbody ID Number
Northwest Region				
Shilshole Bay (Central Puget Sound)	Fish Tissue	Dieldrin	47122G4H1	WA-PS-0240
Springbrook/Mill Creek	Sediment	Bioassay Toxicity	TS53NN	WA-09-1026
Central Region				
Wenatchee River	Fish Tissue	Total PCBs	HM20EV	WA-45-1010
"	"	4,4'-DDT	"	"
"	"	4,4'-DDE	"	"
"	"	4,4'-DDD	"	"
"	"	Alpha-BHC	"	"
Icicle Creek	Fish Tissue	Total PCBs	KN36FW	WA-45-1015
Eastern Region				
Spokane River	Sediment	Bioassay Toxicity	QZ45UE	WA-54-1010

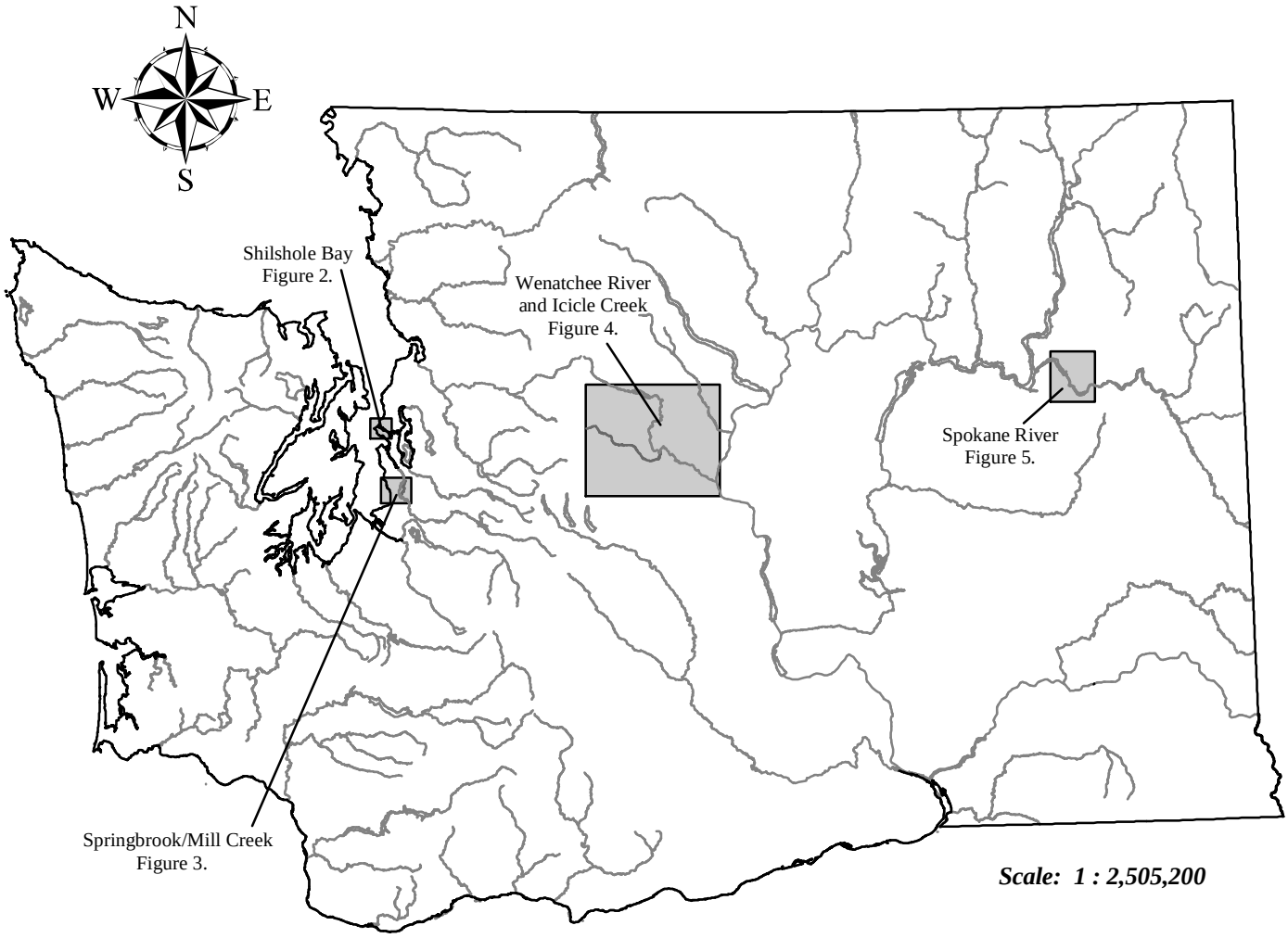

Figure 1. Map of Washington State Showing Major Rivers and Sampling Areas

Northwest Region (Shilshole Bay and Springbrook/Mill Creek)

Shilshole Bay

Shilshole Bay, shown in Figure 2, is located in central Puget Sound at the terminus of the Lake Washington Ship Canal, near the city of Seattle. It is located within Water Resource Inventory Area (WRIA) 8.

Muscle tissue from a single composite of English Sole (*Parophrys vetulus*) from Shilshole Bay was found to have concentrations of dieldrin above the National Toxics Rule (NTR) Human Health Criterion of 0.65 ng/g, parts per billion (ppb) wet weight (1.0 ng/g vs. 0.65 ng/g). These data were found in 1988, as part of an environmental conditions survey conducted by the U.S. Environmental Protection Agency (EPA) (Crecelius et al., 1989). Dieldrin is an insecticide that was phased out of commercial use starting in 1974 and banned completely by 1987 (EPA, 1992). Dieldrin is considered by EPA to be a probable human carcinogen.

Springbrook/Mill Creek

Springbrook/Mill Creek (Figure 3) is a tributary of the Green River and is located in the city of Kent, within WRIA 9. Mill Creek is a headwater tributary of Springbrook Creek, and therefore is often referred to as Springbrook/Mill Creek. The 303(d) listing is located on Mill Creek.

Toxicity was found in the bed sediments of Mill Creek, in a study conducted by Landau Associates in 1992 (Landau Associates, Inc., 1993). Toxicity was measured through the use of bioassay tests. The sediment toxicity study was a part of the larger clean-up effort at the Western Processing Superfund Site, initiated in 1983. The Western Processing company operated a chemical waste processing and recycling facility on its 13-acre site from 1961 to 1983. Some of the chemicals that were cleaned up from soil and water at the site include metals, polychlorinated biphenyls (PCBs), phenols, and volatile organic compounds (EPA, 2000a).

Several studies conducted on Mill Creek sediments have indicated that the main sources of chemical contamination to creek sediments from the Western Processing site are metals, particularly zinc, cadmium, chromium, nickel, copper, arsenic, and lead (Landau Associates, Inc., 1993; Converse GES, 1988, 1989; EPA, 1982, 1984). Polynuclear aromatic hydrocarbons (PAHs), fluoranthene in particular, have been found in Mill Creek sediments both upstream of and adjacent to the Western Processing site, suggesting a source of contamination other than Western Processing (Landau Associates, Inc., 1993). Studies conducted by the Department of Ecology concluded that low dissolved oxygen and degraded fisheries habitat were prevalent in Mill Creek, both adjacent to and well-upstream of Western Processing, also indicating that water quality problems in Mill Creek have sources other than the Western Processing Cleanup site (Yake, 1985; Kittle 1985).

In 1993, as part of the Superfund clean-up effort, bed sediments from Mill Creek were dredged and replaced with new gravel. The stream banks were also stabilized with plantings (EPA, 2000a).

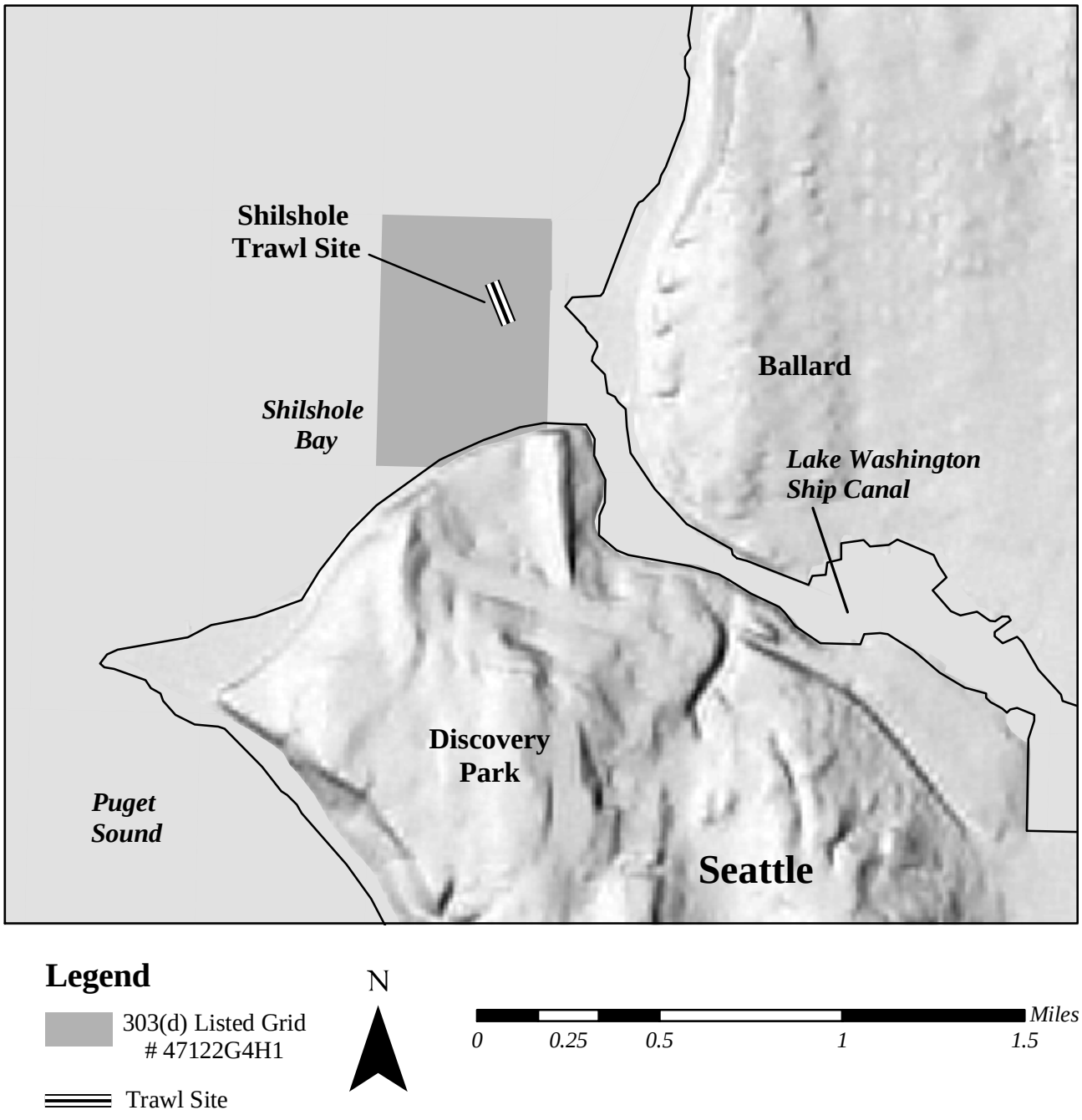

Figure 2. Shilshole Bay Study Area

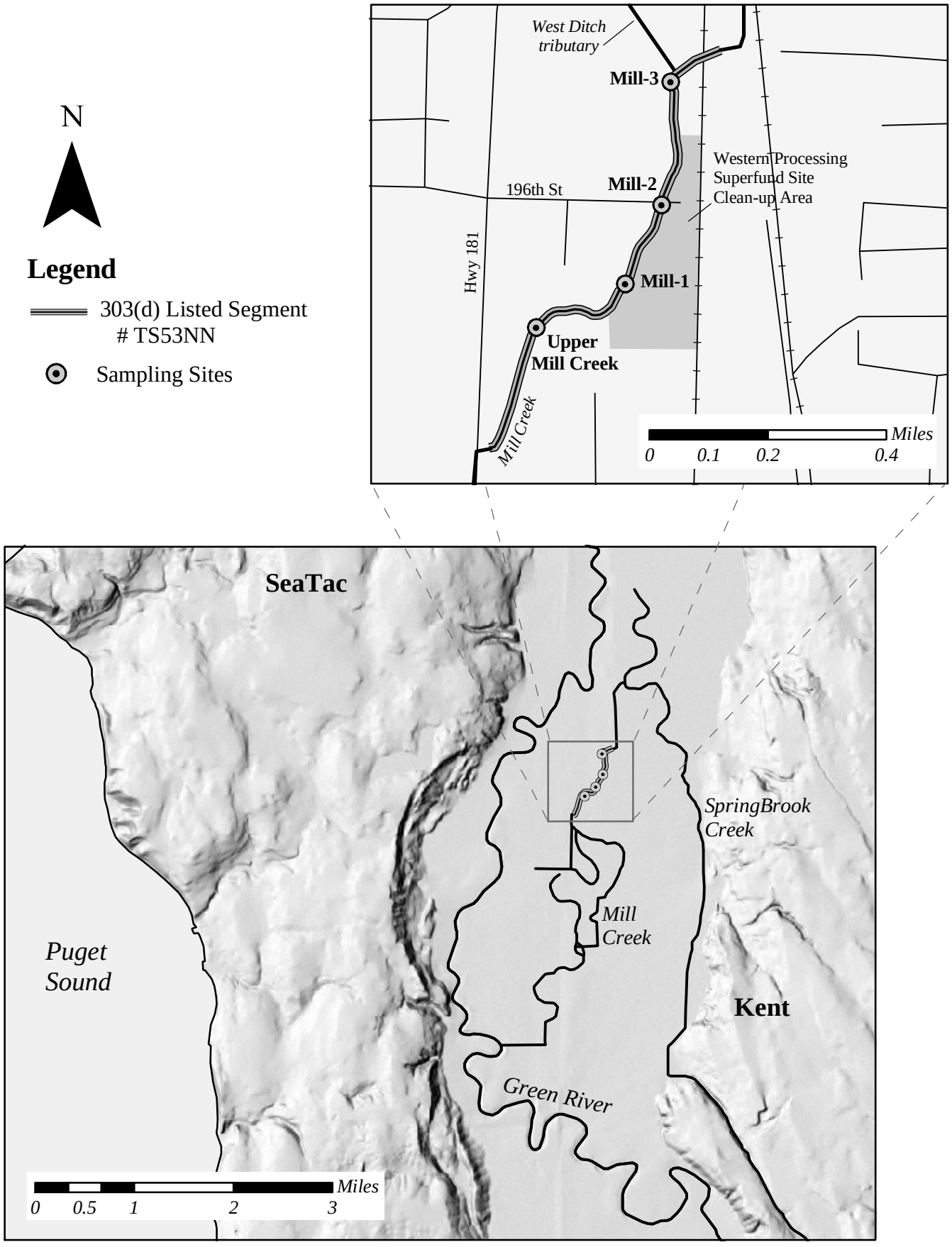

Figure 3. Springbrook/Mill Creek Study Area

Central Region (Wenatchee River and Icicle Creek)

The Wenatchee River and its tributary, Icicle Creek, are shown in Figure 4. They are both located in WRIA 45. Four previous studies have documented the presence of chlorinated chemicals in the Wenatchee River basin. The results of these studies are discussed below and shown in Table 2:

- One of the first studies to identify the presence of chlorinated chemicals in the Wenatchee River was conducted by Ecology in 1984 (Hopkins et al., 1985), in which several chlorinated compounds (total PCBs, alpha-BHC, and DDT) from a single mountain whitefish (*Prosopium williamsoni*) muscle fillet tissue composite exceeded the NTR Human Health Criteria. These data are the basis for the 303(d) listings in the Wenatchee River.

- In 1993, Ecology found elevated DDT and PCB levels in whole largescale sucker (*Catostomus macrocheilus*) composites from the Wenatchee River (Davis et al., 1995).

- In 1997, The United States Geological Survey (USGS) found high concentrations of total PCBs in the water column. Water column concentrations were estimated through the use of semipermeable membrane devices (SPMDs) and were found to be between 200 and 400 times the concentrations found in seven other Columbia River mainstem and tributary sites (USGS, 1999).

- Also in 1997, as part of the Columbia River Basin Fish Contaminant Survey conducted by EPA, several muscle tissue composites of Spring Chinook (*Oncorhynchus tshawytscha*) from Icicle Creek were found to have total PCB concentrations exceeding the NTR Human Health Criteria (EPA, 2002). These data were the basis for the 303(d) listings in Icicle Creek.

DDT and its metabolites (DDD and DDE), and alpha-BHC, are both insecticides that were historically used in agricultural applications. Although EPA banned the use of DDT in 1972 and the use of alpha-BHC in 1977, these chemicals persist in the environment (EPA, 1992). They are considered by EPA to be probable human carcinogens. The presence of these chemicals in the Wenatchee River is likely due to the numerous orchards and other agriculture in the basin.

Sources of PCBs in the Wenatchee River basin are more obscure than the insecticide sources. PCBs were historically used as insulating fluids, plasticizers, pesticide extenders, in inks and carbonless paper, and as heat transfer and hydraulic fluids (EPA, 1992; EPA, 1999). Other research has indicated that DDT can be chemically converted to PCBs via exposure to ultraviolet sunlight (Maugh, 1973). PCBs were also spread by way of recycled waste oil used for dust control and in home and industrial furnaces in some areas of the United States (Chemical Week, 1978). EPA phased out the use and manufacture of PCBs between 1977 and 1985 (EPA, 1992). PCBs are also considered by EPA to be probable human carcinogens.

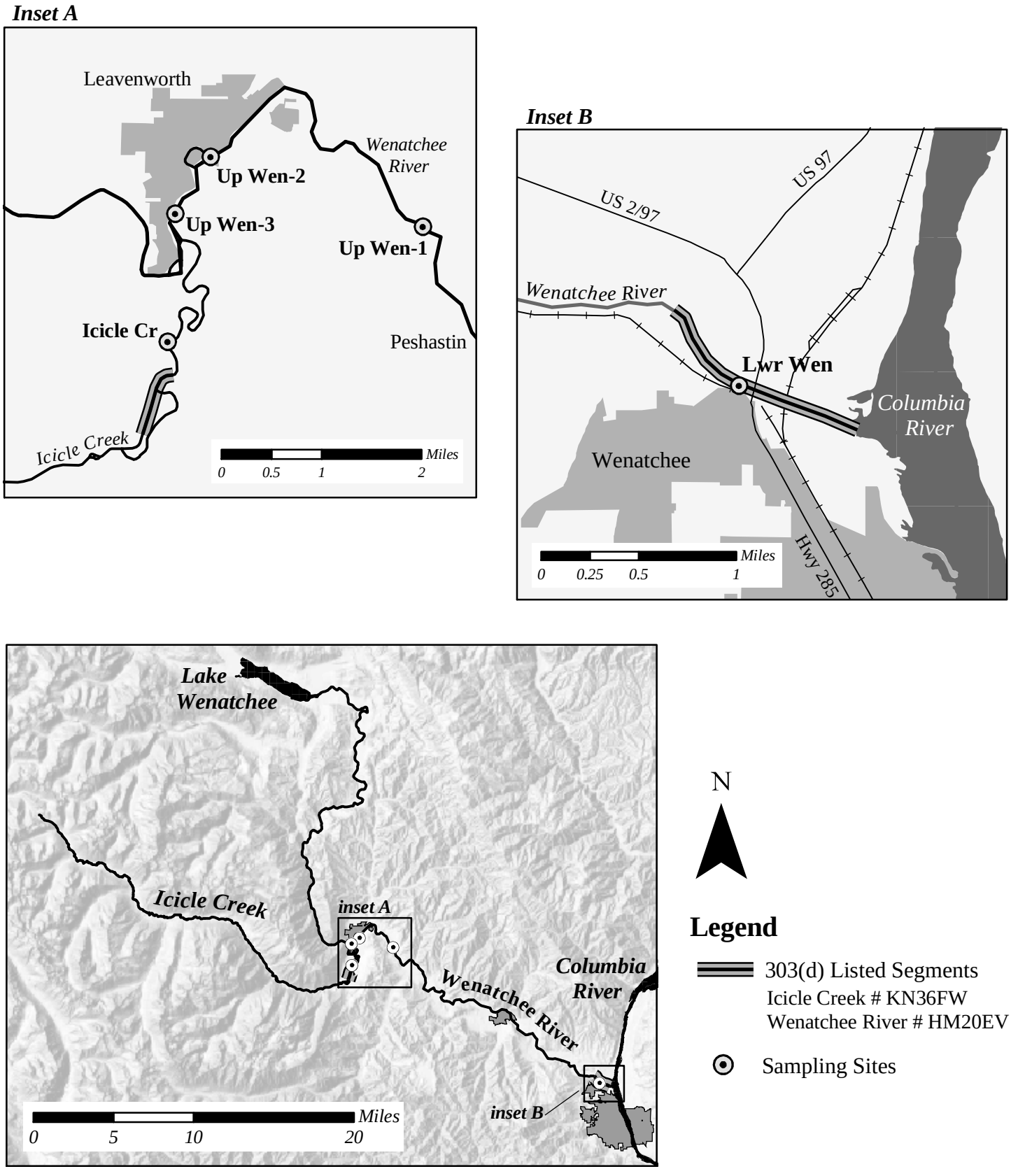

Figure 4. Wenatchee River and Icicle Creek Study Areas

Table 2. Historical Chlorinated Pesticide and PCB Data on Fish Tissue from the Wenatchee River and Icicle Creek, ug/Kg (ppb) wet weight

Location	Wenatchee River		Icicle Creek	National Toxics Rule Criteria*
Date	1984[1]	1993[2]	1997[3]	
Species	Mountain Whitefish	Largescale Sucker	Chinook Salmon	
Tissue	muscle	muscle	whole body	
N =	1 composite	2 composites	3 composites	
4,4'-DDT	250	32/26	--	32
4,4'-DDE	910	380/270	--	32
4,4'-DDD	120	68/47	--	45
Total DDT	1400	494/343	--	32
Alpha-BHC	23	--	--	1.7
PCB-1248	--	170/ --	--	5.3
PCB-1254	--	250/55	13/16/17	5.3
PCB-1260	46	48/49	--	5.3
Total PCBs	46	468/104	13/16/17	5.3

* = Based on EPA bioconcentration factors and water column criteria established under the National Toxics Rule (40 CFR Part 131). Applies to edible tissue only.

[1] = Hopkins et al., 1985

[2] = Davis et al., 1995

[3] = EPA, 2002

Eastern Region (Spokane River)

Bioassay toxicity was documented in sediments from sections of the Spokane River above and below Long Lake Dam in 1994 by Ecology (Batts and Johnson, 1995). Toxicity in the above-dam sections were recently addressed in a study conducted by Ecology in 2000 (Johnson and Norton, 2001). Ecology found toxicity at several of the above-dam sections and has proposed several more listings for the 2002/2004 303(d) List.

Since sediment toxicity has not been addressed since 1994 in the lower section of the Spokane River below Long Lake Dam, it was decided to evaluate current conditions. The 303(d)-listed section of the Spokane River below Long Lake Dam is located near Porcupine Bay (Figure 5) in WRIA 54, an area flooded by the backwater of Franklin D. Roosevelt Lake. Suspected causes of sediment toxicity in this area include zinc and lead (Batts and Johnson, 1995).

There is currently a Total Maximum Daily Load (TMDL) in effect for zinc, cadmium, and lead in the Spokane River (Pelletier, 1998), and a TMDL for PCBs in the Spokane River is currently being conducted (Jack et al., 2003). Pollution sources to the river have been well researched and documented, especially in regards to metals contamination from upper watershed historic mining activities.

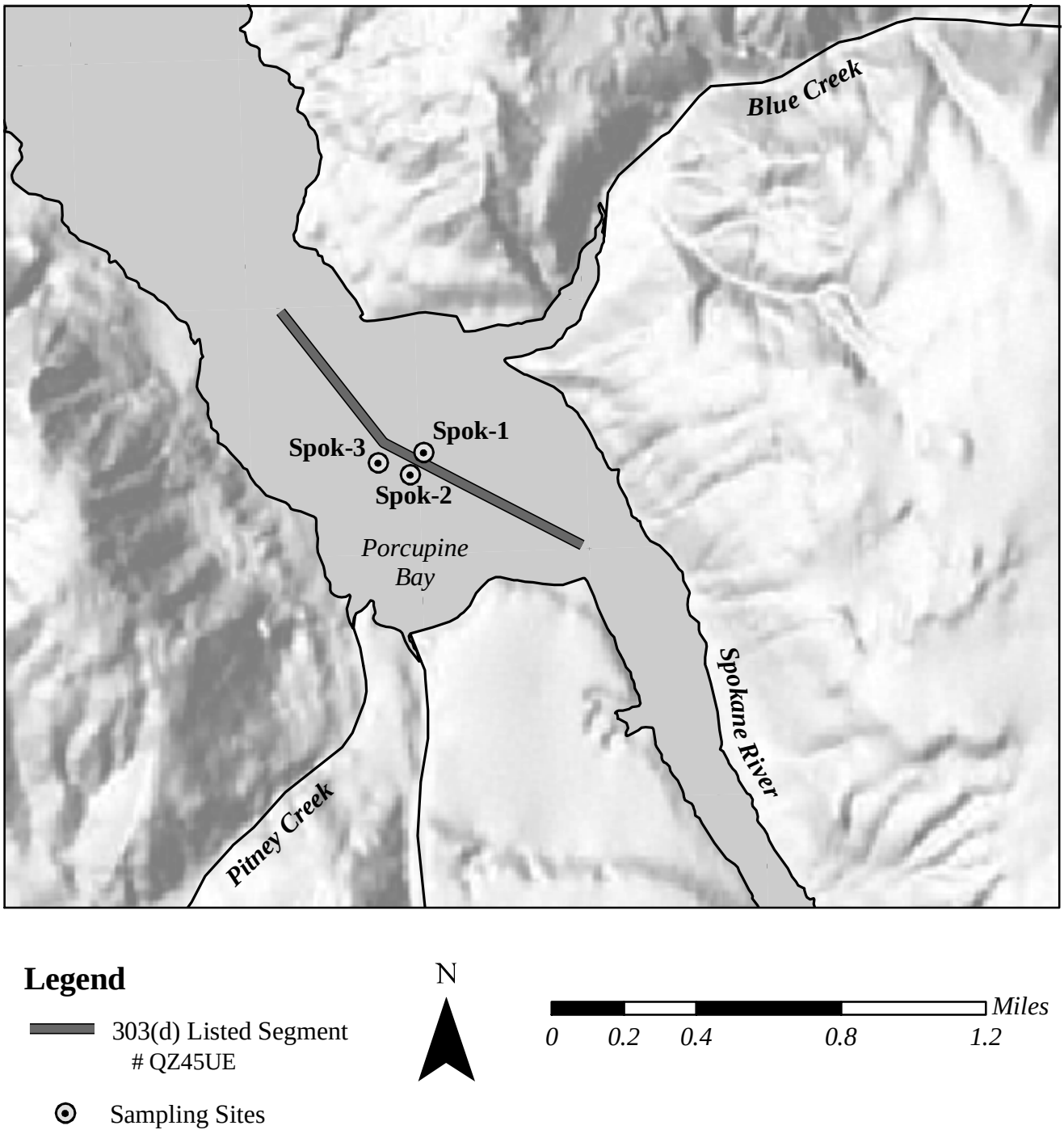

Figure 5. Spokane River Study Area

Methods

Sampling Design

The general design for sampling each 303(d)-listed waterbody was to sample as close as possible to the locations of the previous studies. A detailed description of the study design is present in the Quality Assurance (QA) Project Plan for this study (Era-Miller, 2003).

Wenatchee River and Icicle Creek

The Wenatchee River and Icicle Creek were the exceptions to the basic sampling design described above. For the Wenatchee River, an effort was made to sample different sections of the river to see if there were any differences in contaminant concentrations. Mountain whitefish and suckers were targeted for the following reasons:

- In order to represent the contaminant conditions unique to the Wenatchee River, resident species were needed. Both of these species are known to have low-ranging migratory patterns (Hildebrand, 1991; Viola, 2003).
- Mountain whitefish represent fish that humans are likely to consume, as they are one of the few legal fisheries in the Wenatchee River basin (Viola, 2003).
- EPA recommends analyzing both bottom-feeding species and predator species when screening for contaminants in fish tissue (EPA, 2000b). Suckers are considered bottom-feeders, and mountain whitefish are considered predators.
- Both species have also shown a tendency to accumulate persistent chlorinated chemicals (Davis et al., 1995).

Fishing locations were limited due to difficulties with accessing the river by boat and land, weather conditions such as flooding, and seasonal species availability. Fish were caught in Icicle Creek near the city of Leavenworth, the upper Wenatchee River near the city of Leavenworth and town of Peshastin, and the lower Wenatchee River near the city of Wenatchee.

Field Procedures and Sample Preparation

Sediment

Sediment samples were collected with stainless steel grab samplers following PSEP protocols (PSEP, 1996). Samples from Mill Creek were collected with a 0.02 m^2 petite Ponar by wading into the stream and taking grabs from upstream-directional transects. Samples from Buffalo Lake (reference site) and the Spokane River were collected from an Ecology boat using a 0.05m^2 large Ponar. Sampling locations were recorded using a Magellan GPS unit. Sampling location information can be found in Appendix B, Table B-1.

Each sediment sample consisted of at least three individual grabs. Grabs were deemed acceptable and used when not over-filled with sediment, overlying water was present but not turbid, the sediment surface was undisturbed, and the desired depth of penetration was achieved. Detailed sediment sample descriptions are shown in Table B-2.

Sediments grabs were taken from the top 0-10 cm of sediment (the biologically active zone) and removed from each grab with a stainless steel spoon and placed in a large stainless steel bowl. Sediments touching the sidewalls of the grab were not taken. Once the replicate grabs were collected, sediments were then homogenized by stirring.

Homogenized sediments were then placed in glass jars with Teflon lid liners, cleaned to EPA QA/QC specifications (EPA, 1990). Sample containers, preservation, and holding times are shown in Table B-3. Sediment samples were placed on ice immediately after collection. Samples were refrigerator-stored at Ecology's Operation Center upon return from the field and were then transported to Manchester Laboratory; no more than five days passed between the first sediment collection and arrival at the lab. Manchester then shipped the bioassay samples to the contract laboratories. Chain-of-custody was maintained.

Stainless steel implements used to collect and manipulate the sediments were cleaned by washing with Liquinox detergent, followed by sequential rinses with hot tap water, 10% nitric acid, and deionized water. The equipment was then air-dried and wrapped in aluminum foil. Sediment grabs were cleaned between sites by thoroughly brushing with on-site water.

Fish Tissue

All required state and federal permits were obtained prior to fish collection. English sole were trawl caught from Shilshole Bay by Washington State Department of Fish and Wildlife (WDFW). Fish from the Wenatchee River were caught by a combination of hook and line from shore and with Ecology's electrofishing boat. Fish from Icicle Creek were collected by hook and line from a drift boat. Trawling transects and fishing locations were recorded by GPS and can be found in Appendix B, Table B-1.

Fish captured for analysis were humanely killed with a sharp blow to the head, given an ID number, weighed, and measured. Fish measurement information is located in Table B-4.

Specimens were individually wrapped in heavy aluminum foil, placed in plastic bags, and kept on ice while in the field. Fish were then placed in a freezer at Ecology's Headquarters building immediately upon return from the field.

Preparation of fish tissue samples followed EPA (2000b) guidance. Precautions were taken to minimize contamination during sample processing. Nitrile gloves and aprons were worn, work surfaces were covered with heavy grade aluminum foil, and gloves, aluminum foil, and dissection tools were changed between each composite sample. All resecting instruments were washed thoroughly with Liquinox detergent, followed by sequential rinses of hot tap water, de-ionized water, pesticide-grade acetone, and pesticide-grade hexane.

Samples for analysis were prepared by partially thawing the fish to remove foil wrapper and rinsing in deionized water to remove adhering debris. Fish were de-scaled by knife. For English sole and mountain whitefish, the entire skin-on muscle fillet from one or both sides of each fish was removed with stainless steel scalpels for processing. For largescale suckers, a skin-on muscle fillet from one side of each fish was processed for analysis. The remaining largescale sucker carcasses were also processed for analysis (the carcass chemical concentrations were later used to make mathematical estimations of whole-body concentrations). Tissue samples were homogenized by three passes through a Kitchen-Aid or Hobart food processor.

Fish tissue composite samples were made up of equal-portioned aliquots from five fish. Composite samples were homogenized to uniform color and consistency and placed in jars specifically cleaned for chemical analysis (Table B-3) and sent to Manchester Environmental Laboratory for analysis.

The sex of each fish was recorded during processing. Aging structures (scales, otoliths, opercles, and/or dorsal spines) were removed and sent to the WDFW Laboratory in Olympia, Washington for aging analysis.

Laboratory Analysis

The target analytes, analytical methods, reporting limits, and laboratories that conducted the analyses for both the sediment and fish tissue samples are shown in Table 3.

Table 3. Target Analytes, Analytical Methods, Reporting Limits, and Laboratories

Target Analyte	Reporting Limits	Analytical Method & Method Reference	Laboratory
Sediment			
Microtox Bioassay	n/a	Ecology Protocol (Ecology, 2003)	AMEC Earth & Environmental
Hyalella 10-day Bioassay	n/a	ASTM E-1706 and Method 100.1 (EPA, 2000c)	Northwestern Aquatic
Chironomus 20-day Bioassay	n/a	Method 100.5 (EPA, 2000c)	Northwestern Aquatic
Grain Size[1]	0.1 %	Sieve & Pipet (PSEP, 1996)	Analytical Resources
Total Organic Carbon (TOC)	0.1 %	Combustion/CO2 - Measurement @ 70°C Method 9060	Manchester
Cadmium, Copper, and Lead	0.1 mg/Kg, dry	ICP/MS EPA 200.8	Manchester
Mercury	0.005 mg/Kg, dry	CVAA EPA 245.5	Manchester
Zinc	5.0 mg/Kg, dry	ICP/MS EPA 200.8	Manchester
Fish Tissue			
Chlorinated Pesticides[2]	10-100 ug/Kg, wet	EPA 3540/3620/3665 (prep) EPA 8081	Manchester
PCB Aroclors	50-5000 ug/Kg, wet	EPA 3540 (prep) EPA 8082	Manchester
Percent Lipids	0.1 %	Extraction EPA 608.5	Manchester

[1] = Gravel, sand, silt, and clay fractions

[2] = 4,4'-DDT, 4,4'-DDE, 4,4'-DDD, alpha-BHC, and dieldrin

Sediment Bioassay Methods

The Microtox® test, a chronic toxicity test, measures light emitted by the bioluminescent marine bacteria *Vibrio fischeri* upon exposure to test sediment porewater for 5 and 15 minutes. Results are compared for statistical significance against the results of control and reference sediment porewater. The method for this test is an Ecology modification of PSEP protocols (Ecology, 2003). The Microtox® analysis was performed by AMEC Earth & Environmental, Northwest Bioassay Laboratory, in Fife, Washington.

The Hyalella test, an acute toxicity test, measures the growth and survival of the amphipod *Hyalella azteca* after a 10-day exposure to test sediment (EPA, 2000c). Results are statistically compared to both control and reference sediments.

The Chironomus test, a chronic toxicity test, measures the growth and survival of the midge *Chironomus tentans* after a 20-day exposure to test sediment. The method is a modification of a 50- to 65-day life cycle test developed by EPA (EPA, 2000c). Results are also statistically compared to both control and reference sediments. Both the Hyalella and Chironomus analyses were performed by Northwestern Aquatic Sciences, Newport, Oregon.

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Data Quality

Sediment

Laboratory quality control (QC) samples for the sediment chemistry analyses included method blanks, laboratory duplicates, laboratory control standards, and matrix spikes. All laboratory QC samples were within established QC limits, with the exception of the matrix spike recoveries for lead and zinc. One of the matrix spike recoveries for lead was greater than acceptance limits, indicating that the aliquots from the sample were not homogeneous. The native sample concentration for zinc was an order of magnitude higher than the spike amount, masking any measurable differences between the source sample and spike recovery concentrations. As a result of these matrix spike problems, laboratory bias for lead and zinc could not be evaluated.

Target and actual laboratory measurement quality objectives (MQO) for precision, bias, and accuracy established by the QA Project Plan for the present study are shown in Appendix C, Table C-1.

A field duplicate was analyzed for both the Spokane River and Mill Creek sediments. The relative percent difference (RPD) between field duplicate pairs provide estimates of total variability and overall precision for the sediment chemistry data by accounting for the natural variability (heterogeneity) inherent in the sediments, how well sediment collection and processing procedures were followed in the field, and the quality of the laboratory analysis. Sediment RPD values are shown in Table C-2.

These RPD values indicate good to excellent overall precision for sediment chemistry results. Precision was higher for Spokane River (0-5%) conventionals (TOC, solids, and grain size) than they were for Mill Creek (1-25%). This is likely due to the differences in the homogeneity among sediment grabs at each site. Spokane River sediments were similar among grabs at each site, while Mill Creek sediments were more variable. Metals values for the Spokane River sediments ranged from 6% to 11% RPD.

Sediment Bioassays

Laboratory quality assurance (QA) and QC data for the bioassay tests were carefully reviewed and deemed acceptable by the author of this study. The data were also independently reviewed by Peter Adolphson of Ecology's Toxic Cleanup Program Sediment Management Unit and considered to be acceptable for the purposes of this current study. Detailed QA reviews for the bioassay data are shown in Appendix D.

Sediment bioassay results for Springbrook/Mill Creek and the Spokane River were compared to laboratory negative control samples instead of reference samples for toxicity determination. For Springbrook/Mill Creek, an acceptable reference could not be found prior to sampling, and therefore no reference sample was analyzed. A reference site, Buffalo Lake, was selected for the

Spokane River, but sediments from the lake did poorly on both the *Chironomus tentans* survival and Microtox bioassays. The specific cause of toxicity in the reference sediments is uncertain, but laboratory analysis did show high TOC content, low solids, and high (biochemical oxygen demand) BOD in the bioassay positive control tests. These sediment qualities may be natural for this lake, considering its depth and the fact that it is a sink with no drainage outlets.

Fish Tissue

Laboratory QC samples for fish tissue chemistry included method blanks, laboratory duplicates, laboratory control standards, matrix spikes, and surrogate recoveries. All of the laboratory QC samples were within established QC limits, with the exception of some of the surrogate recoveries and calibration on one of the analytical instrument columns.

PCB aroclor results were qualified as estimates by the laboratory, due to difficulty matching the PCBs found in the test samples to the various possible aroclor patterns. This problem is not uncommon for aroclor analysis in fish tissue, as aroclors are patented mixtures of PCB congeners that separate into their individual congeners in the environment. Congeners are then metabolized at different rates by fish, and the resulting PCBs in fish tissue often do not easily match the patented aroclor mixtures. In addition to the aroclor matching problem, there were difficulties with one of the analytical instrument columns that led to a subset of the DDT data also being qualified as estimates. Dieldrin in two of the Shilshole Bay fish tissue samples (03548111 and 03518113) was reanalyzed to achieve lower reporting limits. All of these QC issues are explained further in the laboratory case narrative for the organics analysis (Appendix C).

Target and actual laboratory measurement quality objective (MQO) values for precision, bias, and accuracy established by the QA Project Plan for the present study are shown in Appendix C, Table C-1. The MQOs for precision were met for most of the samples. The range of values for bias and accuracy for chlorinated chemicals in Table C-1 represent the range of recovery values for the three surrogate recovery chemicals (tetrachloro-m-xylene [TMX], dibutylchlorendate [DBC], decachlorobiphenyl [DCB]) analyzed with each sample. The average percent recovery for these chemicals was 25% for TMX, 60% for DBC, and 100% for DCB, respectively. DCB was the only surrogate recovery chemical that passed laboratory QC limits in all of the samples. The low bias and accuracy values in Table C-1 were calculated from these DCB recoveries and are all well within the MQO targets for bias and accuracy.

A certified standard reference material (SRM)[1] was analyzed to determine the accuracy of the dieldrin and DDT concentrations obtained by the laboratory (Table 4). Accuracy is measured here as the percent difference from the true value. With the exception of 4,4'-DDT, the results appear to be biased low, indicating that the true concentrations in the environment may be higher than study concentrations show.

[1] National Institute of Standards & Technology (NIST) SRM 1946 – Lake Superior Fish Tissue

Table 4. Percent Difference of Standard Reference Material and Present Study Fish Tissue Concentrations for Dieldrin and DDT analogs (ug/Kg ww)

Parameter	Study Value	SRM Value	% Difference
Dieldrin	23	33	-34
4,4'-DDE	270	373	-32
4,4'-DDD	12	18	-38
4,4'-DDT	45	37	+19

A field duplicate (split) sample was analyzed for Shilshole Bay, Wenatchee River, and Icicle Creek. These field duplicates are split samples from the same fish tissue composite samples. RPD values for each of the duplicate pairs (detected analytes only) are shown in Table C-2. These RPD values give estimates of total variability and overall precision for the fish tissue chemistry data by accounting for the natural variability fish tissue, how well tissue processing procedures were followed, and the quality of the laboratory analysis. Compared to other fish tissue studies conducted by Ecology, the overall precision for fish tissue results from the present study is very good.

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Results and Discussion

Sediment

Decision Criteria for Listing of Freshwater Sediment

In order to determine whether the waterbodies listed for sediment toxicity should remain on the 303(d) List, the sediment data had to meet the listing criteria of Ecology's Water Quality 303(d) Listing Policy 1-11 (Ecology, 2002). The criteria used for determining toxicity in freshwater sediment for the current study is as follows:

For biological assessment of freshwater sediment, the 303(d) listing policy states that potential listings will be based on biological tests done in accordance with adopted narrative standards, on a case-by-case basis, in concurrence with the Sediment Management Standards WAC 173-204-340 (Ecology, 2002). Standard Ecology practice for freshwater biological assessment has been the use of a suite of bioassay tests that include both acute and chronic tests. Bioassay tests for this study were chosen from the Sediment Sampling and Analysis Plan Appendix, a guidance document for the Sediment Management Standards (Ecology, 2003). For each listed waterbody, three separate sites were tested and compared for significant statistical difference to both reference and control sediments. Statistical difference, as defined by the Sediment Management Standards, is determined using a t-test with a significance of 0.05.

Table 5 lists the five categories included in the 303(d) List and the definitions for each category. Category 5 is the actual 303(d) List.

Table 5. 303(d) List Water Quality Assessment Categories (Ecology, 2002 draft)

Not impaired or not known to be impaired	
Category 1. Meets Tested Standards	EPA approval and TMDL not required
Category 2. Water of Concern	
Category 3. No Data	
Impaired	
Category 4. Impaired But Does Not Require a TMDL 4a. Has a TMDL 4b. Has a Pollution Control Plan 4c. Impaired by a Non-Pollutant	EPA approval and TMDL not required
Category 5. The 303(d) List	EPA approval and TMDL required

TMDL – Total Maximum Daily Load

Springbrook/ Mill Creek (Northwest Region)

Sediments from four locations along the 303(d)-listed section of Mill Creek were sampled (see Figure 3). The Upper Mill Creek site is located upstream of the Western Processing Superfund clean-up area. The other three sample sites are located on or under the influence of the Western Processing property. Moving in a downstream direction from the Upper Mill Creek site, the sampling locations are Mill-1, Mill-2, and Mill-3.

The results of the Bioassay tests are shown in Table 6. Toxicity (defined here as a statistically significant difference between the test sediment and the laboratory negative control by a t-test with significance of 0.05) was not found in any of the samples for the 10-day *Hyalella azteca* survival bioassay. In stark contrast, toxicity was found in all samples for the 20-day *Chironomus tentans* survival and growth bioassays. The 5- and 15-minute Microtox bioassays showed toxicity only to the Upper Mill Creek site. Due to toxicity in all four Mill Creek samples, Mill Creek should continue to be listed on the 303(d) List (Category 5).

Based on results of the *Chironomus tentans* survival and growth and the Microtox bioassays, the Upper Mill Creek site showed greatest toxicity response. Figure 6 shows a slight downstream trend of lessening toxicity for the Chironomus and Microtox tests in Mill Creek, indicating that the aquatic environment of Mill Creek is most impaired upstream of the Western Processing site. Upstream sources appear to be impacting toxicity in Mill Creek more than the former Western Processing site.

Conventional chemistry results (solids, TOC, and grain size) for Mill Creek are shown in Table 7. Results for all four Mill Creek sites were very similar, with low TOC and sediments composed of mostly sand.

Spokane River (Eastern Region)

Sediments from three clustered locations near the 303(d)-listed section of the Spokane River were sampled (see Figure 5). The locations are Spok-1, Spok-2, and Spok-3. Reference sediments for the Spokane River were taken from Buffalo Lake on the Colville Reservation, about eight miles northeast of Grand Coulee Dam, but were not used as a reference for toxicity determination.

The results of the bioassay tests are shown in Table 6 and Figure 6. As similar to Mill Creek, toxicity was not found in any samples for the 10-day *Hyalella azteca* survival bioassay, and toxicity was found in all samples for the 20-day *Chironomus tentans* survival and growth bioassays. The 5- and 15-minute Microtox bioassays showed no toxicity, except to the reference sediment. Due to the toxicity found in both Chironomus bioassays, the lower Spokane River should remain listed on the 303(d) List (Category 5).

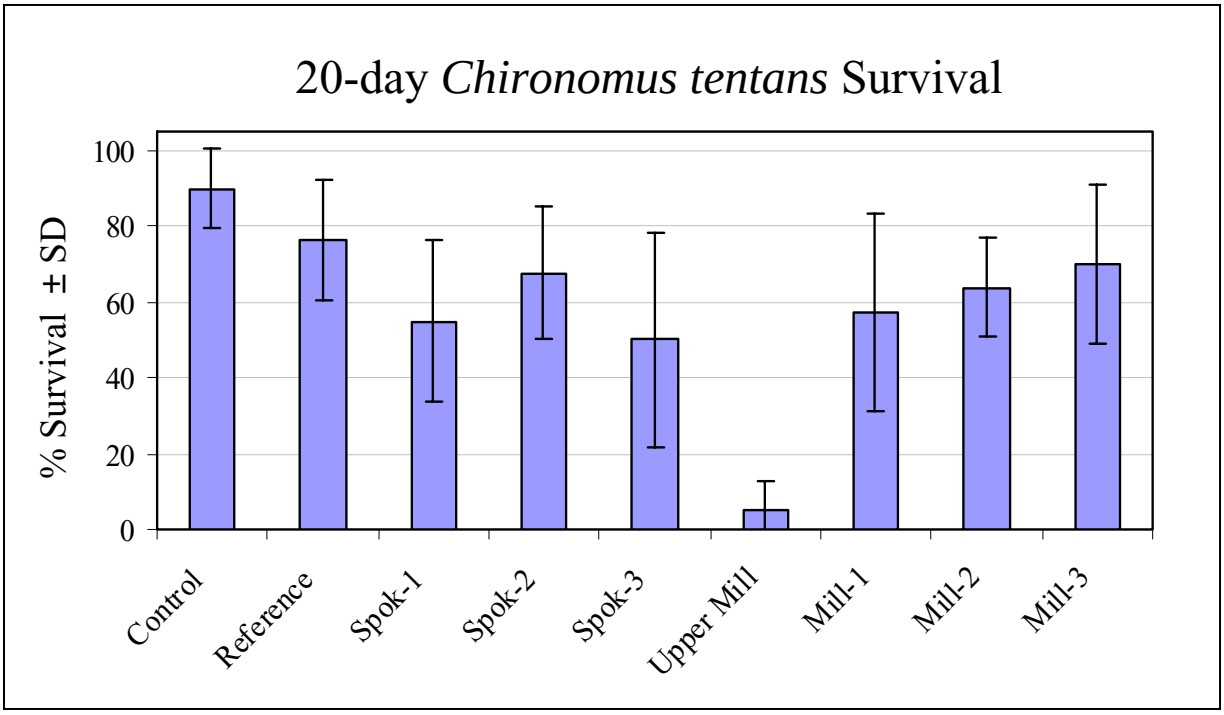
Buffalo Lake should be listed in Category 2 (Waters of Concern) for toxicity to both the Microtox and Chironomus survival tests. Buffalo Lake did not meet the criteria to be listed in Category 5 because only one site was sampled (a minimum of three sites is required). A TMDL is not required for Category 2, but this category allows waterbodies suspected of having contamination to be tracked.

Table 6. Bioassay Results for Mill Creek and Spokane River Sediments

Site name	10-day *Hyalella azteca* (% Survival ± SD)	20-day *Chironomus tentans* survival (% Survival ± SD)	20-day *Chironomus tentans* growth (mg-dry weight ± SD)	5-minute Microtox (percent light output ± SD)	15-minute Microtox (percent light output ± SD)	5-minute Microtox (percent light output ± SD)	15-minute Microtox (percent light output ± SD)
Negative Control	98.8 ± 3.5	90.0 ± 10.7	1.22 ± 0.09	100.5 ± 1.9	98 ± 2.4	97.0 ± 0.5	90.6 ± 0.6
Reference: Buffalo Lake	100.0 ± 0.0	**76.3 ± 16.0**	1.11 ± 0.18	**76.1 ± 6.5**	**74.6 ± 6.5**	**61.3 ± 11.7**	**57.2 ± 11.4**
Spokane River-1	98.8 ± 3.5	**55.0 ± 21.4**	**0.83 ± 0.19**	103.0 ± 1.9	101.7 ± 3.5	--	--
Spokane River-2	96.3 ± 5.2	**67.5 ± 17.5**	**0.82 ± 0.13**	102.3 ± 1.4	99.7 ± 1.4	--	--
Spokane River-3	96.3 ± 5.2	**50.0 ± 28.3**	**0.56 ± 0.33**	102.6 ± 1.4	100.7 ± 1.4	--	--
Upper Mill Creek	96.3 ± 5.2	**5.0 ± 7.6**	**0.06 ± 0.04**	--	--	**73.7 ± 12.3**	**68.0 ± 11.9**
Mill Creek-1	97.5 ± 4.6	**57.5 ± 26.0**	**0.64 ± 0.27**	--	--	97.0 ± 0.8	88.2 ± 2.1
Mill Creek-2	95.0 ± 7.6	**63.8 ± 13.0**	**1.00 ± 0.19**	--	--	97.1 ± 1.2	89.4 ± 0.7
Mill Creek-3	100.0 ± 0.0	**70.0 ± 20.7**	**0.81 ± 0.25**	--	--	97.5 ± 1.5	90.4 ± 1.7

Boxed values indicate statistical significance ($p<0.05$) compared to control sample

-- = Not applicable, Microtox tests were run in two batches



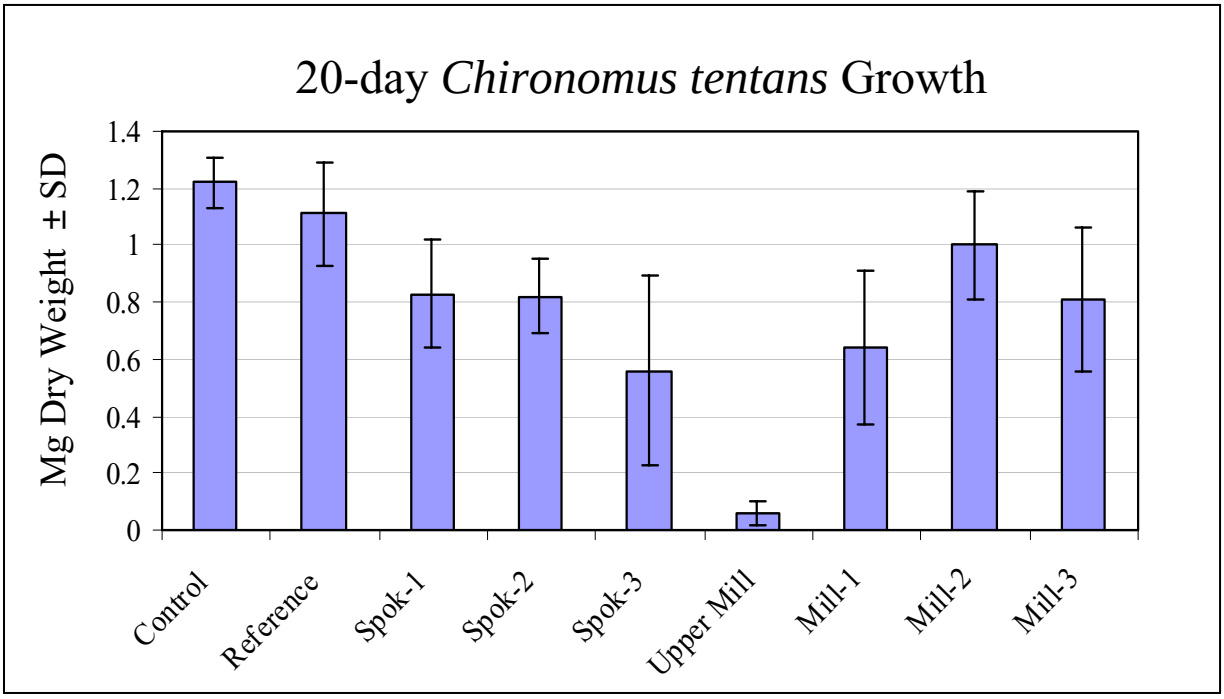
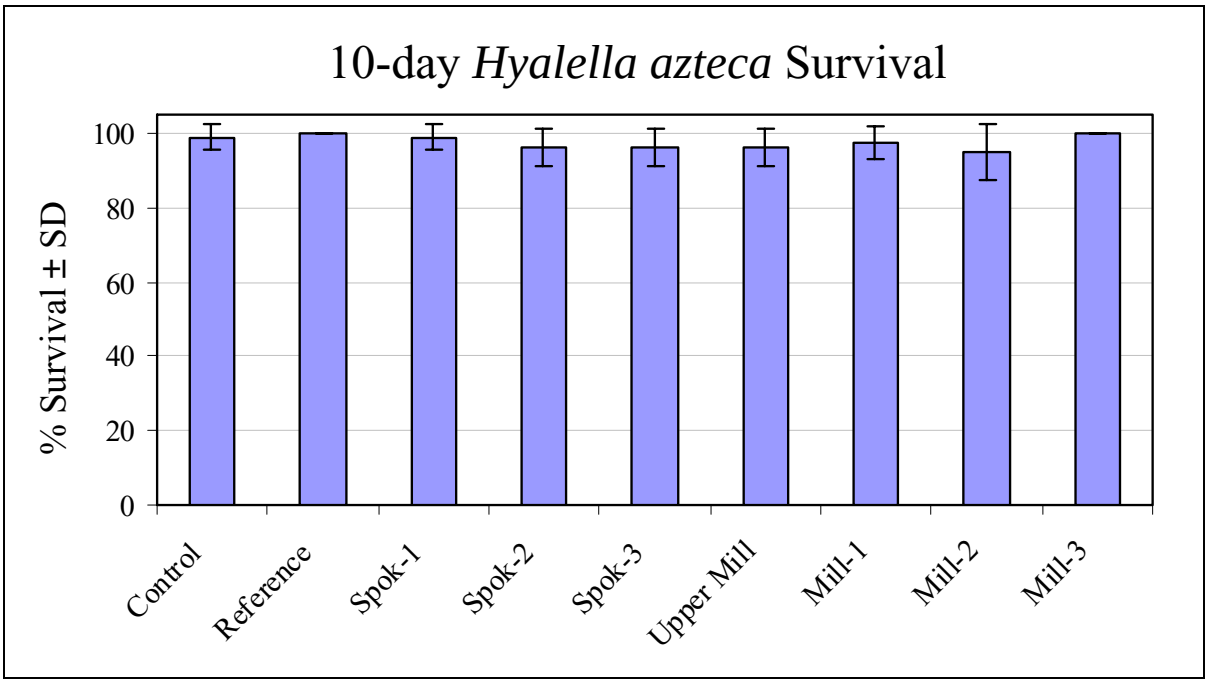


Figure 6. Bar Charts Showing Results for Sediment Bioassay Tests



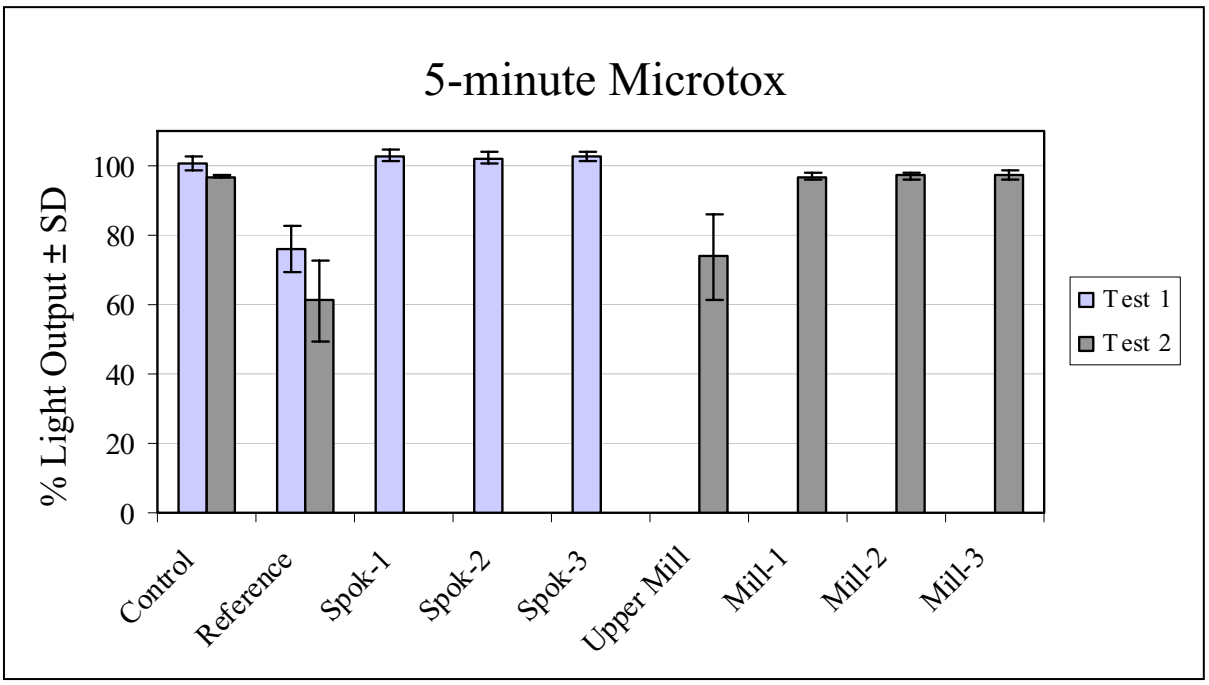


Figure 6 (cont.). Bar Charts Showing Results for Sediment Bioassay Tests

Conventional chemistry results for Buffalo Lake and the Spokane River (solids, TOC, and grain size) are shown in Table 7. Results for the three Spokane River sites are very similar, with low TOC and sediment comprising mostly of fine sediments (silt and clay).

Table 7. Percent Solids, Total Organic Carbon, and Grain Size for Sediments

Site Name	Sample No.	% Solids	% TOC (70° C)	% Grain Size			
				Gravel ≥ 2000µm	Sand < 2000µm – 62µm	Silt < 62µm – 3.9µm	Clay < 3.9µm
Buffalo Lake (reference)	03458103	9.9	8.3	0.3 J	23.3 J	25.4 J	50.9 J
Spokane River-1	03458100	37.1	1.7	0	9.7	66.5	23.8
Spokane River-1 (field duplicate)	03458104	36.1	1.8	0	9.3	66.9	23.8
Spokane River-2	03458101	42.3	1.3	0.4	21.6	57.4	20.6
Spokane River-3	03458102	39.9	1.6	0	6.9	70.3	22.7
Upper Mill Creek	03458108	51.9	1.3	2.7	78.3	16.5	2.6
Mill Creek-1	03458105	67.6	0.8	1.8	79.8	15.0	3.4
Mill Creek-2	03458106	61.9	1.1	4.4	79.9	13.6	2.3
Mill Creek-2 (field duplicate)	03458109	61.2	1.4	3.6	78.9	14.7	2.9
Mill Creek-3	03458107	51.7	1.9	1.5	67.1	27.3	4.1

J = The result is an estimate due to low quantity of fine materials present in the sample

In addition to the conventional chemistry analysis, Buffalo Lake and Spokane River sediments were analyzed for metals (cadmium, copper, lead, mercury, and zinc), and sample Spok-1 was analyzed for PCB congeners. As mentioned previously, zinc and lead were suspected causes of toxicity in the previous study. PCBs were sampled as part of the current Spokane River PCB TMDL study (Jack et al., 2003).

Sediment chemistry results are shown in Table 8. Results in this table are also compared to the Floating Percentile Method (FPM) guideline values; the FPM has recently been recommended for adoption in Washington State because of its reliability in predicting toxicity in freshwater sediments (SAIC and Avocet Consulting, 2003). Reference (Buffalo Lake) sediment concentrations were lower than Spokane sediments for all parameters except mercury; however, all mercury concentrations were still very low. Cadmium and zinc concentrations exceeded FPM values, and therefore could be the cause or contributing factors in the toxicity of the *Chironomus tentans* bioassays for the Spokane sediments.

Table 8. Comparison of Metals and PCB Concentrations in Spokane River and Buffalo Lake Sediments with the Floating Percentile Method Guideline

Site Name	Sample No.	Metals mg/Kg dw (ppm)					Total PCBs µg/Kg (ppb)
		Cadmium	Copper	Lead	Mercury	Zinc	
Buffalo Lake (reference)	03458103	**1.2**	25.9	21.9	0.08	85	3.7 [a]
Spokane River-1	03458100	**5.1**	29.3	82.8	0.05	**874**	12.2 [a]
Spokane River-1 (field duplicate)	03458104	**5.7**	32.6	90.4	0.05	**972**	--
Spokane River-2	03458101	**4.9**	26.4	69.3	0.04	**740**	--
Spokane River-3	03458102	**5.0**	29.2	76.5	0.05	**817**	--
Floating Percentile Method (FPM)	--	0.6	80	335	0.5	140	60 [b]

Bolded values exceed FPM values; FPM values taken from SAIC and Avocet Consulting, 2003

[a] Sum of detected PCB congeners; data from Jack et al., 2003

[b] Sum of detected PCB aroclors

Table 9 gives a comparison between the current sediment chemistry results for the Spokane River (sample Spok-1) and chemistry results from the previous study (Batts and Johnson, 1995). Current concentrations for cadmium, copper, and lead are similar to the previous results, while zinc levels have decreased somewhat. Total PCBs for the two studies were analyzed by different methods (aroclors vs. congeners) making it difficult to determine if PCB levels are decreasing or not.

Table 9. Comparison of Spokane River Sediment Data from 1994 and 2003

Site Name	Sample Date	% fines*	% TOC	Metals mg/Kg dw (ppm)				Total PCBs (ppb)
				Cadmium	Copper	Lead	Zinc	
Spokane River[1]	Aug-94	93	1.8	9.1	33.8	81	1180	35 [a]
Spokane River[2]	Nov-03	91	1.7	5.4	31.0	87	923	12.2 [b]

* Clay and silt fractions (< 62µm)

[1] Batts, D. and A. Johnson, 1994

[2] Present study, Station Spok-1

[a] Sum of detected PCB aroclors

[b] Sum of detected PCB congeners

Observations on Toxicity and the Sediment Bioassays

Assessments of sediment toxicity generally involve a suite of bioassay tests. This is because each bioassay test responds differently to the toxic properties of sediments. For example, bioassays can be responsive to certain types and quantities of chemicals, to a specific exposure pathway (porewater vs. sediment), or time of exposure. The following paragraphs give a qualitative interpretation of the bioassay responses to the test sediments in this study. These interpretations are based on discussions with Peter Adolphson and Brett Betts of Ecology who both have extensive experience with bioassays.

The 10-day *Hyalella azteca* bioassay responded favorably (no toxicity) to all Spokane River and Mill Creek test sediments. This is not an unusual response for Hyalella as it is generally less sensitive than the 20-day *Chironomus tentans* and the 5- and 15-minute Microtox tests, picking up more on the acute rather than the chronic toxic properties of sediments.

Although both considered to be sensitive chronic bioassay tests, the Chironomus and Microtox tests responded very differently to the test sediments. Chironomus showed toxicity to all the Spokane River and Mill Creek sediments, while Microtox demonstrated toxicity only to the Upper Mill Creek sediments. The difference in these responses may be explained by the following reasons:

- The Microtox test uses sediment porewater and not direct exposure to sediments. Chironomus organisms are directly exposed to and may even ingest sediments that might be continually leaching toxicants.

- Chironomus organisms are exposed to sediments for 20 days (vs. Microtox for 5 and 15 minutes). A test of 20 days is more likely to pick up on lower levels of toxicity.

- Because the Microtox organism *Vibrio fischeri* is a marine bacterium, it must be kept in a saline solution during testing. The addition of sodium chloride to the test sediments could have a buffer effect on certain chemicals like metals. If metals were the likely toxicant in the sediments, Microtox's sensitivity to the sediments could be lowered.

Upper Mill Creek is the only test sediment that should be considered acutely toxic, based on the Chironomus and Microtox toxicity responses. It is likely that the cause of this toxicity is due to another type of chemical besides metals (perhaps PAHs). Based on the bioassay responses, established history of metals contamination in both the Spokane River and Mill Creek, and high levels of cadmium and especially zinc in the Spokane sediments from the current study, there is a strong possibility that the chronic toxicity found in the remainder of the samples is the result of metals contamination.

Sediment Bioassay SQS and CSL

Sediment Quality Standards (SQS) and Cleanup Screening Levels (CSL) are bioassay test endpoints used by Ecology's sediment management programs to define toxicity in sediments. Conceptually, SQS represent a level above which minor adverse effects may occur, and CSL represent a level above which significant adverse effects may occur in benthic organisms. SQS and CSL endpoints for freshwater sediment bioassays are shown in Appendix E, Table E-1.

Table E-2 shows bioassay toxicity results for the Spokane River and Mill Creek using statistical difference and SQS/CSL endpoints. SQS and CSL exceedances mirror the toxicity results that are based on statistical difference.

Fish Tissue

Decision Criteria for Listing of Fish Tissue

To determine whether the waterbodies listed for contaminants in fish tissue should remain on the 303(d) List, the fish tissue data had to meet the listing criteria of Ecology's Water Quality 303(d) Listing Policy 1-11 (Ecology, 2002). The criteria used for fish tissue is as follows:

The listing criteria for contaminants in fish include fin fish muscle tissue from at least three single-fish samples or a single composite sample made up of at least five separate fish of the same species. If the average of the three single-fish samples with the highest contaminant concentration or the contaminant concentration of a composite fish sample exceeds criteria for human health impacts, based on EPA's bio-concentration factors and water column criteria established under the National Toxics Rule (NTR), then the waterbody should be listed (Ecology, 2002).

Shilshole Bay (Northwest Region)

The chlorinated chemical results for the two composites of English sole from Shilshole Bay are shown in Table 10 and compared to NTR criteria in Table 11. Although dieldrin was the chemical of interest for Shilshole Bay, the samples were also analyzed for the same set of chemicals as the rest of the fish samples in the present study. Consequently, dieldrin was not detected in the samples, but PCB aroclors 1254 and 1260 were detected and exceeded the NTR criteria. Shilshole Bay should therefore be listed for total PCBs as a new Category 5 listing and de-listed (moved to Category 1) for dieldrin.

The previous total PCB concentration for Shilshole Bay English Sole was 157 ug/Kg (Crecelius et al., 1989). The current total PCB concentrations are an order of magnitude lower at 28 and 48 ug/Kg, suggesting that PCB concentrations may be decreasing.

Table 10. Fish Tissue Chemical Results for Shilshole Bay, Icicle Creek, and the Wenatchee River (October - December 2003)

	Shilshole Bay			Icicle Creek		Upper Wenatchee River		
Station Name	SHILSHOLE	SHILSHOLE	SHILSHOLE	ICICLE CR	ICICLE CR	UP WEN-1	UP WEN-2	UP WEN-2
Sample ID	03518111	03518112 (Replicate)	03518113	03518109	03518110 (Replicate)	03518102	03518103	03518104
Species	Skin-on fillet, English Sole			Skin-on fillet, Mtn Whitefish		Skin-on fillet, Mtn Whitefish		
Mean fish age (yrs)	3.2	3.2	2.4	6.4	6.4	3.6	6.6	2.4
Lipids (%)	0.84	0.88	0.91	4.4	3.89	3.52	4.11	2.62
Organics (ug/Kg ww)								
Alpha-BHC	0.94 U	0.94 U	0.92 U	0.94 U	0.87 U	0.92 U	0.99 UJ	0.97 U
Dieldrin	0.49 UJ	0.94 U	0.5 UJ	0.94 U	0.87 U	0.92 UJ	0.99 UJ	0.97 UJ
4,4'-DDE	0.94 U	0.94 U	0.92 U	**26**	**31**	**37**	**30** J	**47** J
4,4'-DDD	0.94 U	0.94 U	0.92 U	**2.0**	**2.5**	**4.2**	**3.8** J	**3.1**
4,4'-DDT	0.94 U	0.94 U	0.92 U	**3.7**	**5.1**	**13**	**4.8** J	**6.8** NJ
Total DDT	0.94 U	0.94 U	0.92 U	**32**	**39**	**54**	**39** J	**57** J
PCB-1016	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
PCB-1221	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
PCB-1232	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
PCB-1242	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
PCB-1248	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
PCB-1254	**38** J	**38** J	**16** NJ	**35** J	**34** J	**300** J	**43** J	**1200** J
PCB-1260	**10** J	**11** J	**12** J	10 U	9.6 U	**31** J	11 UJ	**89** J
PCB-1262	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
PCB-1268	10 U	10 U	10 U	10 U	9.6 U	10 U	11 UJ	11 U
Total PCBs	**48** J	**49** J	**28** J	**35** J	**34** J	**331** J	**43** J	**1289** J

U = The analyte was not detected at or above the reported result

UJ = The analyte was not detected at or above the reported estimated result

NJ = There is evidence that the analyte is present. The associated numerical result is an estimate.

J = The analyte was positively identified. The associated numerical result is an estimate.

Bold = Detected chemicals

Table 10 (cont.). Fish Tissue Chemical Results for Shilshole Bay, Icicle Creek, and the Wenatchee River (October - December 2003)

	Upper Wenatchee River (cont.)		Lower Wenatchee River			
Station Name Sample ID	UP WEN-3 03518100	UP WEN-3 03518101 (Replicate)	LWR WEN 03518105	LWR WEN 03518105/6	LWR WEN 03518107	LWR WEN 03518108
Species	Skin-on fillet, Mtn Whitefish		Skin-on fillet Lgscale Sucker	Whole body* Lgscale Sucker	Skin-on fillet, Mtn Whitefish	
Mean fish age (yrs)	9	9	14.8	14.8	3.4	1.8
Lipids (%)	4.14	4	1.15	2	4.34	2.58
Organics (ug/Kg ww)						
Alpha-BHC	0.98 U	0.93 U	0.95 U	0.99 U	0.92 U	0.92 U
Dieldrin	0.98 UJ	0.93 UJ	0.95 UJ	0.99 UJ	0.92 UJ	0.92 UJ
4,4'-DDE	**45**	**39**	**49**	**127**	**220**	**51**
4,4'-DDD	**3.2**	**2.7**	**5**	**16**	**37**	**16**
4,4'-DDT	**15**	**8.8**	**6.5**	**20**	**16**	**6.8**
Total DDT	**63**	**51**	**61**	**163**	**273**	**74**
PCB-1016	11 U	10 U	11 U	11 U	10 U	10 U
PCB-1221	11 U	10 U	11 U	11 U	10 U	10 U
PCB-1232	11 U	10 U	11 U	11 U	10 U	10 U
PCB-1242	11 U	10 U	**53** J	**119** J	10 U	10 U
PCB-1248	11 U	10 U	11 U	11 U	**73** J	**89** J
PCB-1254	**720** J	**720** J	**78** J	**260** J	**200** J	**160** J
PCB-1260	**67** J	**72** J	**11** J	**26** J	**29** J	**18** J
PCB-1262	11 U	10 U	11 U	11 U	10 U	10 U
PCB-1268	11 U	10 U	11 U	11 U	10 U	10 U
Total PCBs	**787** J	**792** J	**142** J	**405** J	**302** J	**267** J

* = Whole body fish tissue concentrations were calculated from fillet and carcass concentrations (both the fillet and remaining carcass were analyzed separately)

U = The analyte was not detected at or above the reported result

UJ = The analyte was not detected at or above the reported estimated result

NJ = There is evidence that the analyte is present. The associated numerical result is an estimate.

J = The analyte was positively identified. The associated numerical result is an estimate.

Bold = Detected chemicals

Table 11. Fish Fillet Chlorinated Chemical Concentrations (ug/Kg ww) Compared to National Toxics Rule Criteria

Station Name	Sample ID	Species	Alpha-BHC	Dieldrin	4,4'-DDE	4,4'-DDD	4,4'-DDT	Total PCBs
SHILSHOLE	03518111	English Sole	0.94 U	0.49 UJ	0.94 U	0.94 U	0.94 U	**48** J
SHILSHOLE (rep)	03518112	English Sole	0.94 U	0.94 U	0.94 U	0.94 U	0.94 U	**49** J
SHILSHOLE	03518113	English Sole	0.92 U	0.50 UJ	0.92 U	0.92 U	0.92 U	**28** J
ICICLE CR	03518109	Mtn Whitefish	0.94 U	0.94 U	26	2	3.7	**35** J
ICICLE CR (rep)	03518110	Mtn Whitefish	0.87 U	0.87 U	31	2.5	5.1	**34** J
UP WEN-1	03518102	Mtn Whitefish	0.92 U	0.92 UJ	**37**	4.2	13	**331** J
UP WEN-2	03518103	Mtn Whitefish	0.99 UJ	0.99 UJ	**30** J	3.8 J	4.8 J	**43** J
UP WEN-2	03518104	Mtn Whitefish	0.97 U	0.97 UJ	**47** J	3.1	6.8 NJ	**1289** J
UP WEN-3	03518100	Mtn Whitefish	0.98 U	0.98 UJ	**45**	3.2	15	**787** J
UP WEN-3 (rep)	03518101	Mtn Whitefish	0.93 U	0.93 UJ	**39**	2.7	8.8	**792** J
LWR WEN	03518105	Lgscale Sucker	0.95 U	0.95 UJ	**49**	5	6.5	**142** J
LWR WEN	03518107	Mtn Whitefish	0.92 U	0.92 UJ	**220**	37	16	**302** J
LWR WEN	03518108	Mtn Whitefish	0.92 U	0.92 UJ	**51**	16	6.8	**267** J
National Toxics Rule (Refers to Fillet Tissue)			1.7	0.65	32	45	32	5.3

U = The analyte was not detected at or above the reported result.

UJ = The analyte was not detected at or above the reported estimated result.

NJ = There is evidence that the analyte is present. The associated numerical result is an estimate.

J = The analyte was positively identified. The associated numerical result is an estimate.

Bolded values exceed criteria

Wenatchee River (Central Region)

Seven composites (35 total fish) of fish were collected from the mainstem Wenatchee River. Four composites were collected from the upper Wenatchee River near the cities of Leavenworth and Peshastin, and three composites were collected from the lower part of the river near the city of Wenatchee. Fishing locations for the upper and lower Wenatchee River are displayed in Figure 4. Upper river sampling locations are Up Wen-1, Up Wen-2, and Up Wen-3, and the lower river sampling site is called Lwr Wen. All sampled fish were mountain whitefish, with the exception of one largescale sucker composite from the lower river.

Results for the chlorinated chemical analysis are shown in Table 10. DDT analogs and PCB aroclors were detected in all samples. Alpha-BHC and dieldrin were not detected in any of the samples. Results are compared to the applicable NTR criteria in Table 11. NTR exceedances include 4,4'-DDE and total PCBs, and as a result, the Wenatchee River should be listed on the 303(d) List (Category 5) for total PCBs and 4,4'-DDE in fish tissue. Chemicals 4,4-DDT, 4,4-DDD, and alpha-BHC should be moved from Category 5 to Category 1.

Several differences between the upper and lower Wenatchee River results were observed. Some of these differences are shown in Table 12. For comparability, only mountain whitefish results were averaged in this table. While total DDT concentrations are higher in the lower Wenatchee by a factor of 3, total PCBs in the upper Wenatchee are twice the levels of the lower portion of the river. The mean age of the upper river samples is 5.4 vs. 2.6 in the lower river; however, no correlations between chemical concentrations and age were found with any of the study samples overall.

PCB aroclor patterns also differed between the upper and lower river samples (see Table 10). Only aroclors 1254 and 1260 were found in the upper river samples (including Icicle Creek), while in addition to aroclors 1254 and 1260, aroclor 1248 was found in the lower river mountain whitefish samples and aroclor 1242 was found in the lower river largescale sucker sample.

These differences between the upper and lower river support the standing theory that the upper river mountain whitefish are endemic to the Wenatchee River system, and that the lower river mountain whitefish are probably influenced by the Columbia River. The chemical concentrations in these upper river fish are most likely representative of the chlorinated chemical concentrations unique to the Wenatchee River system.

Table 12. Mean Detected Chlorinated Chemical Concentrations (ug/Kg ww) in Mountain Whitefish Fillet Composites from Icicle Creek and the Wenatchee River

Location	Icicle Creek	Upper Wenatchee	Lower Wenatchee
No. of composites	1	4	2
Mean fish age (yrs)	6.4	5.4	2.6
Lipids (%)	4.4	3.6	3.5
4,4'-DDE	29	39	136
4,4'-DDD	2	4	27
4,4'-DDT	4	9	11
Total DDT	35	52	173
PCB-1248			81
PCB-1254	35	566	180
PCB-1260		50	24
Total PCBs	35	613	285

Composites = 5 individual fish

Contaminant Trends in the Lower Wenatchee River

Chlorinated chemical trends for the lower Wenatchee River are shown in Table 13. Fish from the same location at the mouth of the Wenatchee River (see Figure 4, inset B) were analyzed in 1984, 1993, and during the current study. Total DDT and each of the DDT analogs show a clear pattern of decreasing concentrations over three decades. Alpha-BHC also appears to be decreasing. PCB trends are difficult to decipher due to lack of consistent analysis of all the aroclors among the three studies. Aroclor 1260 does appear to be decreasing in all three sets of results.

Table 13. Comparison of Chlorinated Chemical Concentrations (ug/Kg ww) in Fish Tissue from Various Ecology Studies for the Lower Wenatchee River, 1984 - 2003

Year	1984[1]	2003[2]	1984[1]	2003[2]	1993[3]	2003[2]	National Toxics Rule Criteria*
Species	Mtn Whitefish		Bridgelip Sucker	Lgscale Sucker	Lgscale Sucker		
Tissue	Fillet Tissue		Fillet Tissue		Whole Body		
N =	1 composite	2 composites	1 composite	1 composite	2 composites	1 composite	
4,4'-DDT	**250**	**11**	**23**	**7**	**29**	**20**	32
4,4'-DDE	**910**	**136**	**190**	**49**	**325**	**127**	32
4,4'-DDD	**120**	**27**	**62**	**5**	**58**	**16**	45
Total DDT	**1280**	**173**	**275**	**61**	**412**	**163**	
Alpha-BHC	**23**	nd (0.92)	**2**	nd (0.95)	na	nd (0.99)	1.7
PCB-1242	na	nd (10)	na	**53**	na	**119**	
PCB-1248	na	**81**	na	nd (11)	**170**	nd (11)	
PCB-1254	na	**180**	na	**78**	**153**	**260**	
PCB-1260	**46**	**24**	**41**	**11**	**49**	**26**	
Total PCBs	**46**	**285**	**41**	**142**	**286**	**405**	5.3

* Based on EPA bioconcentration factors and water column criteria established under the National Toxics Rule (40 CFR Part 131). Applies to edible fish tissue only.
na = not analyzed for
nd = not detected
[1] = Hopkins et al., 1985
[2] = Present study
[3] = Davis et al., 1995
Bold = detected chemicals

Regional Contaminant Concentrations Compared to the Wenatchee River

To give some perspective on the current DDT and PCB concentrations in the Wenatchee River, results from the present study were compared to regional concentrations in Table 14. Mean and median total PCB concentrations for the upper Wenatchee River are measurably higher than mean/median concentrations statewide and in the Spokane River, where elevated PCBs have been well documented (Johnson, 2001). Median PCB concentrations in the upper Wenatchee are also higher than the national median. Mean and median total DDT concentrations in the lower Wenatchee River are moderately higher than the statewide concentrations; however, mean and median 4,4'-DDE (generally the largest component of total DDT in fish tissue) concentrations for the lower Wenatchee River were less than national concentrations.

Table 14. Comparison of Total PCB, 4,4'-DDE, and Total DDT Concentrations (ug/Kg ww) for the Present Study with Other Reported Fish Tissue Data

Region	Investigator	Year	No. of Samples/ sites	Mean	Median	Maximum
Total PCBs						
Upper Wenatchee River[1]	Ecology	2003	4 / 3	613	561	1289
United States[2]	EPA	1986-87	nr / 362	1898	209	23,800
Washington State[3]	Ecology	1992-2001	22 / 14	83	43	720
Spokane River[4]	USGS	1999	52 / 4	286	143	1880
4,4'-DDE						
Lower Wenatchee River[1]	Ecology	2003	3 / 1	107	51	220
United States[2]	EPA	1986-87	nr / 362	300	58	14,000
Total DDT						
Lower Wenatchee River[1]	Ecology	2003	3 / 1	136	74	273
Washington State[3]	Ecology	1992-2001	43 / 29	99	23	901

1 = Present Study; fillet tissue only

2 = National Study on Chemical Residues in Fish (EPA, 1992); includes both fillet and whole fish tissues

3 = Data taken from five Ecology Fish Tissue Monitoring Studies (Davis, D. and A. Johnson, 1994; Davis, D., A. Johnson, and D. Serdar, 1995; Davis, D., D. Serdar, and A. Johnson, 1998; Seiders, K., 2003; Serdar, D., A. Johnson, and D. Davis, 1994); fillet tissue only

4 = United States Geological Survey (adapted from Johnson, 2001); fillet tissue only

nr = not reported

Icicle Creek (Central Region)

One composite of mountain whitefish (*Prosopium williamsoni*) was collected from Icicle Creek. The location where the fish were collected on the Creek is shown in Figure 10.

Chlorinated chemical results are shown in Table 10 and compared to NTR criteria in Table 11. DDT analogs were detected but did not exceed NTR criteria. Total PCBs exceeded the NTR criteria and should be listed on the 303(d) List (Category 5).

PCB concentrations from the Icicle Creek fish tissue composite were lower than PCBs found in the mainstem Wenatchee River during the current study. Total PCB concentrations in the Icicle Creek mountain whitefish composite were twice that of concentrations detected in several Chinook Salmon (*Oncorhynchus tshawytscha)* composites in 1997 (EPA, 2002). Average concentrations were 15 ug/Kg for the salmon vs. 35 ug/Kg for the whitefish. Because Chinook Salmon are anadromous, spending much of their life in the ocean and Columbia River system, and mountain whitefish from Icicle Creek are native to the Icicle Creek/Wenatchee River systems, PCB concentrations from this current study are probably more indicative of the PCBs present in Icicle Creek.

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Conclusions

Several sediment bioassay toxicity and fish tissue chemistry 303(d) listings (Category 5) from the Northwest, Central and Eastern regions of Washington State were re-assessed to determine the appropriateness of each listing. The re-assessment information will help Ecology in updating the current 2002/2004 Section 303(d) List of the Clean Water Act.

Sediment bioassay toxicity in Springbrook/Mill Creek (Northwest Region) and the lower Spokane River (Eastern Region) was confirmed, and both waterbodies were recommended for continued Category 5 listing. Buffalo Lake (Central Region) also had bioassay sediment toxicity but was recommended for Category 2 (*Waters of Concern*).

Some of the fish tissue chemistry listings for Shilshole Bay (Northwest Region) and Wenatchee River and Icicle Creek (Central Region) were recommended for listing, and some were recommended for de-listing (Category 1, *Meets Tested Standards*). Shilshole Bay was recommended to be taken off the 303(d) List for dieldrin, but was recommended for the listing for total PCBs. The Wenatchee River was recommended for listing of total PCBs and 4,4'-DDE and for de-listing of alpha-BHC, 4,4'-DDD, and 4,4'-DDT. Icicle Creek was recommended for listing of total PCBs.

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Recommendations

Recommendations for the individual 303(d) listings for each region are shown in Table 15.

Table 15. Recommended Listing Status for Each of the Current 303(d) Listings

Waterbody Name	Matrix	303(d)-listed Parameter	Waterbody ID No.	Listing Decision Category
Northwest Region				
Shilshole Bay	Fish Tissue	Dieldrin	47122G4H1	Category 1
"	"	Total PCBs*	"	**Category 5**
Springbrook/Mill Creek	Sediment	Bioassay Toxicity	TS53NN	**Category 5**
Central Region				
Wenatchee River	Fish Tissue	Total PCBs	HM20EV	**Category 5**
"	"	4,4'-DDT	"	Category 1
"	"	4,4'-DDE	"	**Category 5**
"	"	4,4'-DDD	"	Category 1
"	"	Alpha-BHC	"	Category 1
Icicle Creek	Fish Tissue	Total PCBs	KN36FW	**Category 5**
Buffalo Lake	Sediment	Bioassay Toxicity*	WA-53-9030	Category 2
Eastern Region				
Spokane River	Sediment	Bioassay Toxicity	QZ45UE	**Category 5**

* New listings

Northwest Region (Shilshole Bay and Springbrook/Mill Creek)

Shilshole Bay

Because Shilshole Bay is the outlet of the Lake Washington Ship Canal, a major industrialized waterway, the presence of PCBs is not surprising. Further investigation on PCBs for Shilshole Bay could include a search for existing data on PCBs in tissue, sediments, and water in the area. PCB concentrations appear to be decreasing over time, making the need for a Total Maximum Daily Load (TMDL) study unnecessary.

Springbrook/Mill Creek

Further investigation is recommended for Mill Creek to pinpoint the cause of the toxicity in sediments and potential sources of contamination to the creek, especially at the Upper Mill Creek site. The unidentified pipes draining into the Upper Mill Creek site should be examined as potential sources of contamination. Sediments and any potential substances from pipes or drainages should be screened for BNAs, PAHs, metals, and any other contaminants commonly found in stormwater runoff.

Central Region (Wenatchee River and Icicle Creek)

A TMDL study for PCBs and DDT is recommended for the Wenatchee River and Icicle Creek. The study should focus on potential sources of PCBs and DDT to the river. The use of Semipermeable Membrane Devices (SPMDs) is recommended as a tool for identifying sources. SPMDs are in situ samplers that accumulate lipophilic chemicals such as PCBs and DDT from water. SPMDs can be deployed for short periods, usually about a month. Several recent chlorinated chemical TMDL studies conducted by Ecology have used them quite successfully. SPMDs also were successfully used in the Wenatchee River in 1997 as part of a USGS study (USGS, 1999).

Even though Buffalo Lake was chosen as the reference site for the Spokane River (Eastern Region), it is located in the Central Region. No further investigation is recommended at this time for Buffalo Lake. Toxicity in lake sediments was likely the result of natural conditions.

Eastern Region (Spokane River)

It is recommended that sediments in the lower Spokane River be reevaluated (perhaps in five years) for toxicity to determine if zinc and other contaminant concentrations will continue to decrease. Further study to measure sediment toxicity could include a bioassessment study of native benthic organisms in the 303(d)-listed section of the river, such as an analysis of species abundance or presence of growth deformities in native species like *Chironomus*.

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Appendices

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Appendix A

303(d) Listings

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Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	PUGET SOUND (CENTRAL)	Listing ID #:	7336
Parameter:	Dieldrin	Township:	
Medium:	Tissue	Range:	
Category:	5	Section:	
Listed 98?:	N	Latitude:	47.675
Listed96?:	N	Longitude:	122.415

Remarks

Basis

Crecelius, et al. 1989 , excursions beyond the criterion in edible fish tissue.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	SPRINGBROOK (MILL) CREEK	Listing ID #:	39530
Parameter:	Sediment Bioassay	Township:	22N
Medium:	Sediment	Range:	04E
Category:	5	Section:	01
Listed 98?:	Y	Latitude:	
Listed96?:	Y	Longitude:	

Remarks

Basis

Data from the Dept. of Ecology SEDQUAL database (stations MILLCRP2*MS139*9201146*9/28/1992*none; MILLCRP2*MS140*9201147*9/28/1992*none; MILLCRP2*MS141*9201148*9/28/1992*none) show a significant response to sediment bioassay from samples tested in 1992.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	ICICLE CREEK	Listing ID #:	20306
Parameter:	Total PCBs	Township:	24N
Medium:	Tissue	Range:	17E
Category:	2	Section:	23
Listed 98?:	N	Latitude:	
Listed96?:	N	Longitude:	

Remarks

Tissue samples are from anadromous or nonresident fish and do not include information on the likely source of the toxic pollutant as it relates to the waterbody segment. Since no evidence is available to connect the pollutant to the segment, it has been placed in the Waters of Concern Category.

Basis

EVS Environmental Consultants (2000) show an excursion beyond the National Toxic Rule criterion from Spring Chinook composite of 5 fillet with skin collected in 1997 at station 51-0 (River Mile 2.8) sample #97250814.

EVS Environmental Consultants (2000) show an excursion beyond the National Toxic Rule criterion from Spring Chinook composite of 5 fillet with skin collected in 1997 at station 51-0 (River Mile 2.8) sample #97250815.

EVS Environmental Consultants (2000) show an excursion beyond the National Toxic Rule criterion from Spring Chinook composite of 5 fillet with skin collected in 1997 at station 51-0 (River Mile 2.8) sample #97250816.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	WENATCHEE RIVER	Listing ID #:	14299
Parameter:	Total PCBs	Township:	23N
Medium:	Tissue	Range:	20E
Category:	5	Section:	28
Listed 98?:	N	Latitude:	
Listed96?:	N	Longitude:	

Remarks

Basis

Hopkins et al. 1985. show excursions beyond the National Toxic Rule criterion in a multiple fish composite of edible tissue of Mountain whitefish samples collected in 1984.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	WENATCHEE RIVER	Listing ID #:	12386
Parameter:	4,4'-DDT	Township:	23N
Medium:	Tissue	Range:	20E
Category:	5	Section:	28
Listed 98?:	N	Latitude:	
Listed96?:	N	Longitude:	

Remarks

Basis

Hopkins et al. 1985. show excursions beyond the National Toxic Rule criterion in a multiple fish composite of edible tissue of Mountain whitefish samples collected in 1984.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	WENATCHEE RIVER	Listing ID #:	12387
Parameter:	4,4'-DDD	Township:	23N
Medium:	Tissue	Range:	20E
Category:	5	Section:	28
Listed 98?:	N	Latitude:	
Listed96?:	N	Longitude:	

Remarks

Basis

Hopkins et al. 1985. show excursions beyond the National Toxic Rule criterion in a multiple fish composite of edible tissue of Mountain whitefish samples collected in 1984.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	WENATCHEE RIVER	Listing ID #:	12388
Parameter:	4,4'-DDE	Township:	23N
Medium:	Tissue	Range:	20E
Category:	5	Section:	28
Listed 98?:	N	Latitude:	
Listed96?:	N	Longitude:	

Remarks

Basis

Hopkins et al. 1985. show excursions beyond the National Toxic Rule criterion in a multiple fish composite of edible tissue of Mountain whitefish samples collected in 1984.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	WENATCHEE RIVER	Listing ID #:	14298
Parameter:	ALPHA-BHC	Township:	23N
Medium:	Tissue	Range:	20E
Category:	5	Section:	28
Listed 98?:	N	Latitude:	
Listed96?:	N	Longitude:	

Remarks

Basis

Hopkins et al. 1985. show excursions beyond the National Toxic Rule criterion in a multiple fish composite of edible tissue of Mountain whitefish samples collected in 1984.

Water Quality Assessment for Washington

2002/2004 Candidate List

Water Body Name:	SPOKANE RIVER	Listing ID #:	39519
Parameter:	Sediment Bioassay	Township:	28N
Medium:	Sediment	Range:	37E
Category:	5	Section:	33
Listed 98?:	Y	Latitude:	
Listed96?:	Y	Longitude:	

Remarks

Basis

Data from the Dept. of Ecology SEDQUAL database (stations SPOKNR94*LRSA*328003*8/10/1994*Spokane River; SPOKNR94*LRSA*328003*8/10/1994*Spokane River; NONE) show a significant response to sediment bioassay from samples tested in 1994.

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Appendix B

Location and Sample Information

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Table B-1. Sediment and Fish Tissue Sampling Location Descriptions

Location Name	Date	Time	Water Depth (ft)	Latitude North	Longitude West	Location Description
Sediment Sites						
Buffalo Lake						
Composite Grab 1	11/5/03	12:45	116	**48° 03.941'**	**118° 53.332'**	East from the boat launch, near lake center (deepest part of lake)
Composite Grab 2	11/5/03	13:00	116	48° 03.915'	118° 53.236'	
Composite Grab 3	11/5/03	13:15	116	48° 03.015'	118° 53.040'	
Spokane River-1						
Composite Grab 1	11/6/03	9:40	85	**47° 53.043'**	**118° 08.982'**	Porcupine Bay, northeast of boat launch (upstream)
Composite Grab 2	11/6/03	10:00	85	47° 53.014'	118° 08.979'	
Composite Grab 3	11/6/03	10:15	84	47° 53.026'	118° 08.977'	
Spokane River-2						
Composite Grab 1	11/6/03	10:45	83	**47° 52.991'**	**118° 09.031'**	Porcupine Bay, northeast of boat launch (upstream)
Composite Grab 2	11/6/03	12:00	70	47° 52.987'	118° 08.974'	
Composite Grab 3	11/6/03	12:10	80	47° 52.950'	118° 09.077'	
Spokane River-3						
Composite Grab 1	11/6/03	12:30	80	**47° 53.021'**	**118° 09.145'**	Porcupine Bay, northeast of boat launch (upstream)
Composite Grab 2	11/6/03	12:40	82	47° 53.005'	118° 09.139'	
Composite Grab 3	11/6/03	12:50	82	47° 53.007'	118° 09.106'	
Upper Mill Creek						
Transect Start	11/8/03	13:50	2.5	**47° 25.408'**	**122° 14.757'**	Upstream of the Western Processing Superfund Site, behind Arco Gas Station
Transect End	11/8/03	15:00	2.5	47° 25.383'	122° 14.720'	
Mill Creek-1						
Transect Start	11/7/03	13:00	2	**47° 25.490'**	**122° 14.617'**	On Western Processing Superfund Site property, near portable office buildings
Transect End	11/7/03	14:40	2	47° 25.474'	122° 14.612'	
Mill Creek-2						
Transect Start	11/7/03	15:35	2	**47° 25.602'**	**122° 14.530'**	On Western Processing Superfund Site property, upstream of 196th St. culvert
Transect End	11/7/03	16:45	2	47° 25.567'	122° 14.526'	
Mill Creek-3						
Transect Start	11/8/03	11:00	2	**47° 25.782'**	**122° 14.542'**	On Western Processing Superfund Site property, upstream of West Ditch Tributary
Transect End	11/8/03	12:00	2	47° 25.777'	122° 14.543'	

Location Name	Date	Time	Water Depth (ft)	Latitude North	Longitude West	Location Description
Fish Tissue Sites						
Shilshole Bay						
Trawl Start	10/9/03	15:57	40	**47° 40.548'**	**122° 24.744'**	Shilshole Bay (200 yards of trawl distance)
Trawl End	10/9/03	16:01	40	47° 40.645'	122° 24.806'	
Lower Wenatchee	11/18/03	15:00	nm	**47° 27.541'**	**120° 20.237'**	Between Hwy 2 bridge and aqueduct bridge
Upper Wenatchee-1	11/14/03	14:30	nm	**47° 34.912'**	**120° 36.802'**	Upstream of Peshastin (off the right bank)
Upper Wenatchee-2	11/18/03	10:00	nm	**47° 35.425'**	**120° 39.485'**	Leavenworth City Park
Upper Wenatchee-3	11/14/03	11:00	nm	**47° 35.100'**	**120° 40.145'**	Leavenworth Golf Course, hole #11 (left bank)
Icicle Creek	12/3/03	11:00	10	**47° 33.936'**	**120° 40.105'**	Downstream of the East Leavenworth Rd. bridge

nm = not measured

Bolded coordinates were used for study locations in Ecology's Environmental Information System (EIM)

Table B-2. Sediment Sample Descriptions

Site Name	Sample No.	Collection Date	Collection Method	Mean Penetration Depth (cm)	Mean Sample Depth (cm)	No. of Grabs in Composite	Sediment Quality Description
Buffalo Lake (reference)	03458103	11/5/03	0.05m^2 Large Ponar	14	10	3	Homogenous dark gray (almost black) fluffy mud with minor amounts of organic hair-like debris throughout
Spokane River-1	03458100/4	11/6/03	0.05m^2 Large Ponar	12	10	3	Homogenous light gray muddy silt with a thin (<1cm) rust-colored top layer
Spokane River-2	03458101	11/6/03	0.05m^2 Large Ponar	12	10	3	Same as Spokane River-1
Spokane River-3	03458102	11/6/03	0.05m^2 Large Ponar	13	10	3	Same as Spokane River-1
Upper Mill Creek	03458108	11/8/03	0.02m^2 Petite Ponar	7	6	5	Dark brown silty sand with moderate amount of organic material and leaves, small dark leach-like worms, and slight diesel smell
Mill Creek-1	03458105	11/7/03	0.02m^2 Petite Ponar	8	7	6	Heterogeneous mix of sand, rock, and clay layers with minor amounts of organic material and a thin (<0.5cm) rust-colored top layer
Mill Creek-2	03458106/9	11/7/03	0.02m^2 Petite Ponar	7	6	5	Same as Mill Creek-1, but with more organic material and leaves
Mill Creek-3	03458107	11/8/03	0.02m^2 Petite Ponar	8	7	5	Homogenous brownish-gray muddy silt with moderate amounts of organic material and leaves

Table B-3. Containers, Preservations, and Holding Times for the Sediment and Fish Tissue Analysis.[1]

Analyte	Container	Preservation	Holding Time
Sediment Bioassays			
Chironomus	1-liter glass jar	Refrigerate, 4° C	2 weeks
Hyalella	1-liter glass jar	Refrigerate, 4° C	2 weeks
Microtox	1-liter glass jar	Refrigerate, 4° C	2 weeks
Sediment Chemistry			
TOC	2-oz glass jar	Refrigerate, 4° C	28 days (1 year if frozen)
Grain Size [2]	8-oz glass jar	Refrigerate, 4° C	6 months
Cadmium, Copper, Lead, Zinc	8-oz glass jar	Refrigerate, 4° C	6 months
Mercury	4-oz glass jar	Refrigerate, 4° C	28 days
Fish Tissue Chemistry			
Chlorinated Pesticides PCB Aroclors Percent Lipids	Certified 4-oz glass Teflon lid liner	Refrigerate, 4° C Freeze, -18° C	7-day Extraction 14-day Analysis (1 year if frozen)

[1] = Information taken from the Manchester Laboratory Manual and PSEP Protocols (MEL, 2003; PSEP, 1996)

[2] = Gravel, sand, silt, and clay fractions

Table B-4. Fish Tissue Sample Biological Information

Location Name	Sample Number	Collection Date	Species	Fork Length (mm)	Total Length (mm)	Weight (g)	Sex	Age (yrs)
UP WEN-3	03518100/01	11/14/03	MTWF	315	340	393	F	5
		11/14/03	MTWF	320	343	388	F	5
		11/14/03	MTWF	380	412	677	F	7
		11/14/03	MTWF	400	430	888	F	12
		11/14/03	MTWF	402	428	879	F	16
			Mean	**363**	**391**	**645**	n/a	**9**
UP WEN-1	03518102	11/14/03	MTWF	335	361	409	F	6
		11/14/03	MTWF	308	337	344	F	5
		11/14/03	MTWF	262	284	184	M	3
		11/14/03	MTWF	224	244	114	F	1
		11/14/03	MTWF	308	332	306	M	3
			Mean	**287**	**312**	**271**	n/a	**3.6**
UP WEN-2	03518103	11/18/03	MTWF	348	373	489	F	7
		11/18/03	MTWF	341	367	425	F	4
		11/18/03	MTWF	362	386	554	F	11
		11/18/03	MTWF	308	331	300	F	5
		11/18/03	MTWF	390	417	599	F	6
			Mean	**350**	**375**	**473**	n/a	**6.6**
UP WEN-2	03518104	11/18/03	MTWF	263	287	220	M	3
		11/18/03	MTWF	288	310	244	F	3
		11/18/03	MTWF	244	265	162	M	2
		11/18/03	MTWF	233	252	162	F	2
		11/18/03	MTWF	223	242	122	M	2
			Mean	**250**	**271**	**182**	n/a	**2.4**
LWR WEN	03518105	11/18/03	LGSC	450	474	1039	F	16
		11/18/03	LGSC	438	464	982	F	16
		11/18/03	LGSC	425	436	874	F	11
		11/18/03	LGSC	451	472	1007	F	15
		11/18/03	LGSC	447	466	936	F	16
			Mean	**442**	**462**	**968**	n/a	**14.8**
LWR WEN	03518107	11/18/03	MTWF	267	289	230	M	3
		11/18/03	MTWF	291	315	233	M	5
		11/18/03	MTWF	265	293	225	M	3
		11/18/03	MTWF	275	299	219	M	3
		11/18/03	MTWF	267	290	224	M	3
			Mean	**273**	**297**	**226**	n/a	**3.4**

Location Name	Sample Number	Collection Date	Species	Fork Length (mm)	Total Length (mm)	Weight (g)	Sex	Age (yrs)
LWR WEN	03518108	11/18/03	MTWF	244	266	142	F	2
		11/18/03	MTWF	218	237	114	nm	1
		11/18/03	MTWF	235	248	153	M	2
		11/18/03	MTWF	228	251	127	M	2
		11/18/03	MTWF	245	268	158	M	2
			Mean	**234**	**254**	**139**	n/a	**1.8**
ICICLE CR	03518109/10	12/3/03	MTWF	280	302	241	F	3
		12/3/03	MTWF	381	412	635	F	7
		12/3/03	MTWF	410	439	840	F	12
		12/3/03	MTWF	359	387	560	F	7
		12/3/03	MTWF	296	324	250	F	3
			Mean	**345**	**373**	**505**	n/a	**6.4**
SHILSHOLE	03518111/12	10/9/03	ESOLE	nm	323	310	F	3
		10/9/03	ESOLE	nm	305	276	F	4
		10/9/03	ESOLE	nm	325	347	F	4
		10/9/03	ESOLE	nm	235	167	M	3
		10/9/03	ESOLE	nm	250	176	F	2
			Mean	nm	**288**	**255**	n/a	**3.2**
SHILSHOLE	03518113	10/9/03	ESOLE	nm	275	149	M	6
		10/9/03	ESOLE	nm	239	132	F	2
		10/9/03	ESOLE	nm	225	110	F	2
		10/9/03	ESOLE	nm	223	111	F	1
		10/9/03	ESOLE	nm	212	90	M	1
			Mean	nm	**235**	**118**	n/a	**2.4**

n/a = not applicable
nm = not measured
MTWF = Mountain Whitefish, *Prosopium williamsoni*
LGSC = Largescale Sucker, *Catostomus macrocheilus*
ESOLE = English Sole, *Parophrys vetulus*

Appendix C

Quality Assurance Information

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Table C-1. Actual and Target Value Comparison for the Laboratory Measurement Quality Objectives (MQOs)

Parameter	Precision (% RSD)*		Bias (% of true value)**		Accuracy (% deviation from true value)***	
	Actual	Target	Actual	Target	Actual	Target
TOC	0.2-1.3	7	nc	nc	nc	nc
Grain Size	0-7.6	10	nc	nc	nc	nc
Cadmium	5.1	7.5	10	5	20	20
Copper	3.9	7.5	5	5	13	20
Lead	6.7	7.5	22	5	35	20
Mercury	0.4	7.5	7	5	8	20
Zinc	5.1	7.5	10	5	20	20
Lipids	4-11	20	nc	nc	nc	nc
4,4'-DDE	3-7	15	6-77	20	13-90	50
4,4'-DDD	2-8	15	6-77	20	10-93	50
4,4'-DDT	2-16	15	6-77	20	11-108	50
PCB-1242	6	15	6-77	20	20-90	50
PCB-1248	13	15	6-77	20	32-103	50
PCB-1254	7-35	15	6-77	20	21-147	50
PCB-1260	6-8	15	6-77	20	19-92	50

* = Precision was calculated from laboratory duplicates except for metals, which were calculated from matrix spike duplicates

** = Bias was calculated from matrix spike recoveries for metals and from surrogate recoveries for organics

*** = Accuracy is a function of both precision and accuracy

nc = not calculated

Table C-2. Precision of Field Duplicates for Sediment and Fish Tissue Results

	Sediment						Fish Tissue								
Station Name	Spokane River-1			Mill Creek-2			Upper Wenatchee-3			Icicle Creek			Shilshole Bay		
Sample Number	3458100	3458104		3458106	3458109		3518100	3518101		3518109	3518110		3518111	3518112	
Parameter	Result	Result	RPD	Result	Result	RPD	Result	Result	RPD	Result	Result	RPD	Result	Result	RPD
Metals (mg/Kg dw)															
Cadmium	5.11	5.69	**11%**	--	--	--	--	--	--	--	--	--	--	--	--
Copper	29.3	32.6	**11%**	--	--	--	--	--	--	--	--	--	--	--	--
Lead	82.8	90.4	**9%**	--	--	--	--	--	--	--	--	--	--	--	--
Mercury	0.051	0.054	**6%**	--	--	--	--	--	--	--	--	--	--	--	--
Zinc	874	972	**11%**	--	--	--	--	--	--	--	--	--	--	--	--
Organics (ug/Kg ww)															
4, 4' -DDE	--	--	--	--	--	--	45	39	**14%**	26	31	**18%**	nd	nd	nc
4, 4' -DDD	--	--	--	--	--	--	3.2	2.7	**17%**	2	2.5	**22%**	nd	nd	nc
4, 4' -DDT	--	--	--	--	--	--	15	8.8	**52%**	3.7	5.1	**32%**	nd	nd	nc
Total DDT	--	--	--	--	--	--	63.2	50.5	**22%**	31.7	38.6	**20%**	nd	nd	nc
PCB-1242	--	--	--	--	--	--	nd	nd	nc	nd	nd	nc	nd	nd	nc
PCB-1248	--	--	--	--	--	--	nd	nd	nc	nd	nd	nc	nd	nd	nc
PCB-1254	--	--	--	--	--	--	720	720	**0%**	35	34	**3%**	38	38	**0%**
PCB-1260	--	--	--	--	--	--	67	72	**7%**	nd	nd	nc	10	11	**10%**
Total PCBs	--	--	--	--	--	--	787	792	**1%**	35	34	**3%**	48	49	**2%**
% lipids	--	--	--	--	--	--	4.14	4	**3%**	4.4	3.89	**12%**	0.84	0.88	**5%**
% TOC (70° C)	1.7	1.8	**5%**	1.1	1.4	**24%**	--	--	--	--	--	--	--	--	--
% solids	37.1	36.1	**3%**	61.9	61.2	**1%**	--	--	--	--	--	--	--	--	--
% gravel	0	0	**0%**	4.4	3.6	**20%**	--	--	--	--	--	--	--	--	--
% sand	9.7	9.3	**4%**	79.9	78.9	**1%**	--	--	--	--	--	--	--	--	--
% silt	66.5	66.9	**1%**	13.6	14.7	**8%**	--	--	--	--	--	--	--	--	--
% clay	23.8	23.8	**0%**	2.3	2.9	**23%**	--	--	--	--	--	--	--	--	--

-- = not analyzed for

nc = not calculated

nd = not detected

RPD = Relative Percent Difference

Manchester Environmental Laboratory
7411 Beach Dr E, Port Orchard, Washington 98366

Case Narrative
April 29, 2004

Subject: 303(D) Verification - Fish Tissue

Samples: 03518100 - 03518113

Case no. 217503

Officer: Brandee Era-Miller

By: John Weakland and Sara Sekerak

Pesticides and PCB Analysis

Analytical Method(s)

The tissue samples were extracted with 50/50 methylene chloride and hexane using a Soxhlet apparatus. The samples were solvent exchanged to hexane then received a 0% and 50% Florisil treatment. The 0% extract was then solvent exchanged to iso-octane and concentrated to 1 mL. The 50% fractioned samples, with exception of the matrix spikes and spike blank, were brought to 9 mL in hexane and of that 4 mLs were archived. The spike blank and matrix spike were brought to 10 mL in hexane. Five milliliters of each of the samples were treated with an acetonitrile back extract. The final 50% extracts were solvent exchanged to iso-octane and concentrated to 1 mL.

All of the 0% samples were acid treated prior to analysis. The 50% fraction samples were split and half were analyzed un-acidified and the other half after acidification.

Holding Times

All samples were prepared and, with exception of additional dilutions made for Aroclor 1254, analyzed within the method holding times.

Calibration

The initial calibrations were acceptable and within established QC limits.

The continuing calibrations checks were acceptable and within established QC limits on at least one column. Results were reported from the column that was within limits with the following

exceptions. Although the lower DBC value was used, the results for samples OCT4041A2 (SRM), 03518100-8103, 8105-8111 were bracketed by high CCVs. Sample results for DBC on samples 03518113 (LDP1) spike and 8113 spike duplicate (LDP2) were reported from data bracketed by low CCVs. The other surrogate, DCB, however was reported from passing CCV data, thus no qualification of these samples was necessary.

Degradation Check

The degradation checks were acceptable and within established QC limits.

Blanks

There were no target analytes detected in the method blanks.

Surrogates

A cocktail containing Tetrachloro-m-xylene (TMX), 4,4-Dibromooctafluorobiphenyl (DBOB), Dibutylchlorendate (DBC) and Decachlorobiphenyl (DCB) was used for the surrogate spiking of each sample and QC sample prior to processing. In addition to the sample extracts, two blanks, one laboratory control sample (LCS) and a standard reference material (1946) sample were prepared using an independently prepared surrogate and spiking solution.

The results for DBC were taken from the 0% fraction. The TMX and DCB results were taken from the 50% acidified fraction.

The percent recoveries of DBC for the QC samples and samples 03518105, 8112 and 8113 were slightly below QC limits and the results were not qualified. The DBC percent recovery for the blanks was also slightly below the established limits. These recoveries for method blanks are often seen and no qualifications were made based upon this.

The percent recovery of TMX in all of the samples and QC were low. However, the other surrogate DCB was within limits and therefore no qualification is necessary.

Matrix Spikes

A stock spiking solution was added to sample 03518113 and 03518113 duplicate. The percent recoveries and relative percent differences were acceptable and within established QC limits.

Laboratory Control Samples (LCS)

The percent recovery of the LCS (OCS4041A1) was acceptable and within established QC limits.

Comments

Because of the matrix interferences from the fish tissue an exact pattern match of the reported Aroclors could not be made. This is attributed to Aroclors being metabolized, or weathered, by the fish. Consequently, the reported Aroclor most closely matched the analytical standard. Because of the uncertainty associated with reporting PCB mixtures in this matrix, all of the Aroclor results have been qualified "J", estimated value.

The manner in which these samples fractionated significantly varied from what was to be expected and resulted in difficulties interpreting the Aroclor patterns. This anomaly has been attributed to inefficient separation resulting from a suspected Florisil lot. Some of the Aroclor 1016 and 1242 data was reported from two peaks instead of the minimum three peaks as required. Because the results were already qualified as estimates no further qualification was necessary. All the Aroclor 1242 results were quantitated using the initial calibration of the very similar Aroclor 1016.

The relative percent difference (%RPD) between columns for Aroclor 1254 on samples 03518105 duplicate, 03518113 and DDT on sample 03518104 was greater than 40%. Consequently the results were qualified as, "NJ", evidence the analyte is present but reported at the estimated value.

Sample 03518103 sustained a loss of about 2 mL of extract during the initial stage of the acetonitrile back extraction. Consequently, all results have been qualified as estimates, either "J" or "UJ".

The data is usable as qualified.

Data Qualifier Codes

U	-	The analyte was not detected at or above the reported result.
J	-	The analyte was positively identified. The associated numerical result is an estimate.
UJ	-	The analyte was not detected at or above the reported estimated result.
REJ	-	The data are unusable for all purposes.
NAF	-	Not analyzed for.
N	-	For organic analytes there is evidence the analyte is present in this sample.
NJ	-	There is evidence that the analyte is present. The associated numerical result is an estimate.
NC	-	Not Calculated
E	-	The concentration exceeds the known calibration range.
bold	-	The analyte was present in the sample. (Visual Aid to locate detected compounds on report sheet.)

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Manchester Environmental Laboratory

7411 Beach Dr E, Port Orchard, Washington 98366

Case Narrative

June 16, 2004

Subject: 303(D) Verification - Fish Tissue, Supplemental Report

Samples: 03518111, 03518113 Re-extracted

Officer: Brandee Era-Miller

By: M. Mandjikov

Dieldrin Analysis

Analytical Method(s)

Each tissue sample was extracted into methylene chloride and hexane (50/50 v/v) using a Soxhlet apparatus. After extraction the extracts were solvent exchanged into hexane and eluted through a Florisil® column with 100% hexane which was collected and archived. The Florisil column was then eluted with a 50% preserved diethyl ether/hexane solution. The 50% Florisil fractions of the extracts were back extracted with acetonitrile to remove interferences. They were each concentrated to 1 mL and eluted through a micro Florisil cartridge with the 50% diethyl ether/hexane solution. The 50% fractions of the extracts were solvent exchanged to iso-octane and concentrated to 1mL.

The extracts were analyzed using dual column GC-ECD. These methods are modifications of EPA SW- 846 methods 3540, 3620, and 8081.

Holding Times

All samples were prepared and analyzed within the method holding times.

Calibration

The initial calibration curves for all analytes are acceptable and within the established QC limits.

All initial calibration verification (ICV) standards are acceptable and within established QC limits.

All sample results were bracketed with continuing calibration verification (CCV) standards that were acceptable and within established QC limits.

Instrument Degradation of Endrin and DDT

The degradation of Endrin in the first degradation control sample exceeded the control limit by 2%. The closing degradation control sample was within the established control limits. Dieldrin does not degrade to the extent that Endrin does and all the CCVs are within the control limits, therefore, the Endrin degradation has little effect upon the Dieldrin results.

Blanks

There were no target analytes detected in the method blanks.

Surrogates

Each sample, blank and QA sample were spiked Dibutylchlorendate (DBC). All the surrogate recoveries are below 50% with the exception of sample 03518111. All Dieldrin results are qualified as not detected at the estimated reporting limit, "UJ".

Spiked Samples

Sample 03518113 was prepared in triplicate. Two of the replicates were spiked with the 100 ng of Dieldrin. One spiked sample was lost during processing. The Dieldrin recovery is within the established QC limits.

Laboratory Control Sample

The percent recovery of Dieldrin was below the established QC limits. All Dieldrin results are qualified due to the low surrogate recoveries and no further qualification is necessary.

Comments

In an effort to concentrate the sample and reduce interferences, the micro-Florisil technique was performed on these extracts after back extracting with acetonitrile. The laboratory is currently in the process of evaluating this cleanup technique for the tissue matrix. The poor surrogate and LCS recoveries are a result of using the method before it has been optimized. However, there is no evidence of Dieldrin present in either sample at levels that would challenge the 0.65 ug/Kg requirement assuming a 100% recovery of the sample extract. All Dieldrin results are qualified as not detected at and estimated reporting limit, "UJ", due to the low QC recoveries in this project.

Appendix D

Bioassay Data

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Toxicological Evaluation of Sediments for Verification of 303(d) Listed Sites

Microtox® 100 Percent Sediment Porewater Analysis

Prepared for
Washington State Department of Ecology
Manchester Laboratory
7411 Beach Drive East
Port Orchard, WA 98366-8204

Prepared by
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December 2003

INTRODUCTION

As part of an environmental program being conducted by Washington State Department of Ecology, toxicity tests were conducted on freshwater sediment collected from sites in rivers located in Eastern Washington. Sediment toxicity tests were performed using the bacterium *Vibrio fischeri* to assess toxicity in 100 percent sediment porewater using the Microtox® assay. This report documents the results of these tests.

METHODS

Samples

Sediment samples were collected on 10 November 2003. The sediments were identified as SPOK-1, SPOK-2, SPOK-3, SPOK-REF, MILL-1, MILL-2, MILL-3, and MILL-REF. Sediments were sampled into 1-L glass jars and were transported in a cooler. The cooler was filled with ice and transported to AMEC by WDOE personnel on 12 November 2003. Appropriate chain-of-custody procedures were employed during transportation and evaluation of samples.

Upon arrival at AMEC, the cooler was opened and the contents inspected and compared to documentation provided on the chain-of-custody forms. The temperature was measured in the cooler. Samples were held in the dark at 4±2°C prior to initiation of the tests.

The sediment samples were not sieved prior to analysis.

Test Procedure

The luminescent marine bacterium *V. fischeri* was used as the test organism for the Microtox® test. The bacteria were exposed to porewater extracted from sediment samples and light readings were measured after a 5 minute incubation period and then after an additional 5 minutes and 15 minutes of exposure. Test equipment included the Microtox Model 500 Analyzer, which measures light output and is equipped with a 15°C chamber to maintain test temperature in the samples and a 4°C chamber to keep the rehydrated bacteria chilled.

The tests were conducted in accordance with WDOE (2003) test protocol and summarized in Table 1. Approximately 50 milliliters (mL) of porewater was extracted

from each sample by centrifuging for 30 minutes at 4500 G. Each porewater extract was adjusted to a salinity of 20 parts per thousand (ppt) with Forty Fathoms artificial seasalt. The dissolved oxygen in each sample was between 50 and 100 percent saturation and, consequently, the samples did not require aeration. The pH was adjusted to 7.8 to 8.2 using NaOH or HCl (Appendix Table B-1). The control was deionized water adjusted to 20 ppt with artificial seasalt.

Tests were conducted using five replicates. Disposable glass cuvettes were placed in the Microtox test wells and 1 mL of salinity adjusted porewater was added. The rehydrated bacteria (reagent) were thoroughly mixed and 10 microliters (µL) was added to each test cuvette. After an initial incubation period of 5 minutes, the control cuvette was placed in the read chamber of the Microtox Analyzer to set the instrument. Initial light readings (I_0) were then taken by placing each cuvette in the read chamber of the Microtox Analyzer and measurements were recorded on a data sheet. Light output was measured in each cuvette after an additional 5 minutes (I_5) and 15 minutes (I_{15}) of exposure. Test acceptability criterion is mean control final light output greater than 72% of initial output.

A reference toxicant test using phenol was conducted in conjunction with the sediment porewater test to ensure that the sensitivity of the test was within the acceptable range of historical values determined in this laboratory.

Table 1. Summary of testing conditions for the Microtox® test.

Test date	1 December 2003
Test organism source	Strategic Diagnostics
Batch number and expiration date	Lot # 3B2159, Expiration date 03/05
Control	Salt water (20 ppt) prepared with 40 Fathoms Sea Salts
Sample preparation	Centrifugation at 4500 G for 30 minutes; salinity adjustment to 20 ppt using 40 Fathoms Sea Salts; pH adjustment to 7.8 – 8.2 with HCl or NaOH
Test chamber	Glass cuvette
Test volume	1 mL
Volume of inoculum/replicate	10 µL
Number of replicates/sample	5
Test temperature	15 ± 1°C
Aeration	None
Reference toxicant	Phenol

Statistical Analyses

Statistical analyses were performed using GraphPad Prism software, Version 3.0. Multiple comparison procedures using a one-tailed t-test were used to assess differences between the control and each sample.

RESULTS

A summary of results for the Microtox assay is provided in Appendix A. Water quality data, reference toxicant data, and chain-of-custody information are contained in Appendices B, C, and D, respectively.

Mean luminescence of the reference sediment (SPOK-REF) was significantly reduced compared to the control in both tests and was not used in evaluation of any of the test sediment porewaters.

Light ouput by MILL-REF sediment was 76% and 75% of control ouput at I_5 and I_{15}, respectively (Appendix Table A-1). Additionally, mean light output for MILL-REF was statistically reduced compared to the control.

Light output for all other test sediments was greater than 90% of control output at both I_5 and I_{15}.

Table 2. Mean and standard deviation light reduction in Microtox® tests.

Sample	Percent of initial light output I_5	p-values	I_{15}	p-values
Test 1				
Control	100.5 ± 1.9	---	98.0 ± 2.4	---
SPOK-REF	**76.1 ± 6.5**	**<0.001***	**74.6 ± 6.5**	**<0.001***
SPOK-1	103.0 ± 1.9	---	101.7 ± 3.5	---
SPOK-2	102.3 ± 1.4	---	99.7 ± 1.4	---
SPOK-3	102.6 ± 1.4	---	100.7 ± 1.4	---
Test 2				
Control	97.0 ± 0.5	---	90.6 ± 0.6	---
SPOK-REF	**61.3 ± 11.7**	**0.001***	**57.2 ± 11.4**	**0.001***
MILL-REF	**73.7 ± 12.3**	**0.007***	**68.0 ± 11.9**	**0.006***
MILL-1	97.0 ± 0.8	---	88.2 ± 2.1	0.03*
MILL-2	97.1 ± 1.2	---	89.4 ± 0.7	0.23*
MILL-3	97.5 ± 1.5	---	90.4 ± 1.7	0.33*

Note 1: Tests were conducted in 3 sets due to time constraints in the test design. The control and reference sample (Spok-Ref) were included in each set.

Note 2: Bolded data indicates test ouput of less than 90% of Control AND statistically significant adverse effects relative to the control ($p<0.05$).

Note 3: Statistical analysis conducted using one-tailed t-test. * indicates unequal variance. Welch's correction applied.

QA/QC

The temperatures of the samples received on 12 November 2003 were 6°C. The containers were received in good condition and the documentation provided on the chain-of-custody forms matched the labeling of the test containers themselves. Copies of the chain-of-custody forms are provided in Appendix D.

Control performance met the acceptability criterion of greater than 72 percent of initial light output at the 5 and 15-minute readings.

The LC_{50} estimate from the reference toxicant test was 20.0 mg/L phenol after 5 minutes of exposure and 23.4 mg/L phenol after 15 minutes of exposure. These values are within the historical sensitivity of mean $\pm$ 2 standard deviations for previous tests conducted in this laboratory (Appendix C).

References

American Society of Testing and Materials (ASTM). 1996. Standard Test Method for Assessing the Microbial Detoxification of Chemically contaminated Water and Soil Using a Toxicity Test with a Luminescent Marine Bacterium. ASTM Designation D 5660-96.

AZUR Environmental. 1998. MicrotoxOmni Test Manual.

GraphPad Software Inc. 1994-2000. GraphPad Prism, Version 3.0.

Tidepool Scientific Software. 1992-1994. ToxCalc Comprehensive Toxicity Data Analysis and Database Software, Version 5.0.

Washington State Department of Ecology. 2003. Microtox 100 Percent Porewater Toxcity Assessement (Final 01/15/03).

APPENDIX A

RESULT SUMMARIES

Appendix Table A-2. Microtox 100 Percent Sediment Porewater Test
303(d) Verification - Sediment Bioassay Study
Washington State Department of Ecology
Test Date: 21 November 2003

Site	Light Reading Replicate							$T_{(mean)}/R_{(mean)}$	$T_{(mean)}/C_{(mean)}$	Change in light readings compared to initial control $I_{(t)(mean)}/I_{(0)C(mean)}$	Change in light readings compared to final control $I_{(t)(mean)}/I_{(t)C(mean)}$	Evaluation of initial light output $I_{(0)(mean)}/I_{(0)C(mean)}$
	Reading	1	2	3	4	5	Mean					
Control	$I_{(0)}$	96	92	86	88	75	87					
	$I_{(5)}$	93	89	83	86	73	85			0.97		
	$I_{(15)}$	86	84	78	80	68	79			0.91		
	$C_{(5)}$	0.97	0.97	0.97	0.98	0.97	0.97					
	$C_{(15)}$	0.90	0.91	0.91	0.91	0.91	0.91					
Spok-Ref	$I_{(0)}$	68	47	63	46	59	57					0.65
	$I_{(5)}$	66	44	59	42	57	54				0.63	
	$I_{(15)}$	61	40	56	39	54	50				0.63	
	$R_{(5)}$	0.76	0.50	0.68	0.48	0.65	0.61					
	$R_{(15)}$	0.70	0.46	0.64	0.45	0.62	0.57					
Mill-Ref	$I_{(0)}$	84	57	59	62	72	67					0.76
	$I_{(5)}$	81	55	57	60	69	64					
	$I_{(15)}$	75	50	53	54	65	59					
	$T_{(5)}$	0.93	0.63	0.65	0.69	0.79	0.74	1.20	0.76			
	$T_{(15)}$	0.86	0.57	0.61	0.62	0.74	0.68	1.19	0.75			
Mill-1	$I_{(0)}$	89	70	75	91	75	80					0.92
	$I_{(5)}$	86	67	73	89	73	78					
	$I_{(15)}$	77	60	68	81	67	71					
	$T_{(5)}$	0.97	0.96	0.97	0.98	0.97	0.97	1.58	1.00			
	$T_{(15)}$	0.87	0.86	0.91	0.89	0.89	0.88	1.54	0.97			
Mill-2	$I_{(0)}$	69	74	89	73	83	78					0.89
	$I_{(5)}$	66	72	88	71	80	75					
	$I_{(15)}$	61	66	80	66	74	69					
	$T_{(5)}$	0.96	0.97	0.99	0.97	0.96	0.97	1.58	1.00			
	$T_{(15)}$	0.88	0.89	0.90	0.90	0.89	0.89	1.56	0.99			
Mill-3	$I_{(0)}$	84	90	82	80	69	81					0.93
	$I_{(5)}$	81	90	79	78	67	79					
	$I_{(15)}$	74	82	74	72	64	73					
	$T_{(5)}$	0.96	1.00	0.96	0.98	0.97	0.97	1.59	1.00			
	$T_{(15)}$	0.88	0.91	0.90	0.90	0.93	0.90	1.58	1.00			

$I_{(0)}$ is the light reading after the initial five minute incubation period

$I_{(5)}$ is the light reading five minutes after $I_{(0)}$

$I_{(15)}$ is the light reading fifteen minutes after $I_{(0)}$

$C_{(t)}$, $R_{(t)}$, and $T_{(t)}$ are the changes in light readings from the intial reading in each sample container for the control, reference sediment and test sites. $I_{(t)}/I_{(0)}$

Quality Control Steps:

1. Is control final mean output greater than 72% control initial mean output?

   $I_{(5)}$: $F_{c(mean)}/I_{c(mean)}$=97%

   $I_{(15)}$: $F_{c(mean)}/I_{c(mean)}$=91%

Yes. Control results are acceptable.

2. Does the reference final mean exceed 80% of control final mean?

   $I_{(5)}$: $F_{R(mean)}/F_{C(mean)}$=63%

   $I_{(15)}$: $F_{R(mean)}/F_{C(mean)}$=63%

No. Reference site results are not acceptable to be used in statistical analyses.

3. Is the reference initial mean > 80% of control initial mean?

   $I_{R(mean)}/I_{C(mean)}$=65%

No. Reference initial mean should not be used to calculate change in light readings at $I_{(5)}$ and $I_{(15)}$ for reference site.

4. Are test initial mean values > 80% of control initial mean values?

   Mill-Ref: $I_{T(mean)}/I_{C(mean)}$=76%, use initial mean output to calculate change in light readings.

   Mill-1: $I_{T(mean)}/I_{C(mean)}$=92%, use to calculate change in light readings.

   Mill-2: $I_{T(mean)}/I_{C(mean)}$=89%, use to calculate change in light readings.

   Mill-3: $I_{T(mean)}/I_{C(mean)}$=93%, use to calculate change in light readings.

Appendix Table A-1. Microtox 100 Percent Sediment Porewater Test
303(d) Verification - Sediment Bioassay Study
Washington State Department of Ecology
Test Date: 21 November 2003

Site	Light Reading	Replicate						$T_{(mean)}/R_{(mean)}$	$T_{(mean)}/C_{(mean)}$	Change in light readings compared to initial control $I_{(t)(mean)}/I_{(0)C(mean)}$	Change in light readings compared to final control $I_{(t)(mean)}/I_{(t)C(mean)}$	Evaluation of initial light output $I_{(0)(mean)}/I_{(0)C(mean)}$
	Reading	1	2	3	4	5	Mean					
Control	$I_{(0)}$	98	93	94	87	88	92					
	$I_{(5)}$	96	96	94	87	89	92			1.00		
	$I_{(15)}$	95	95	92	84	85	90			0.98		
	$C_{(5)}$	0.98	1.03	1.00	1.00	1.01	1.00					
	$C_{(15)}$	0.97	1.02	0.98	0.97	0.97	0.98					
Spok-Ref	$I_{(0)}$	69	60	74	73	72	70					0.76
	$I_{(5)}$	69	60	75	73	73	70				0.76	
	$I_{(15)}$	67	59	74	73	70	69				0.76	
	$R_{(5)}$	0.75	0.65	0.82	0.79	0.79	0.76					
	$R_{(15)}$	0.73	0.64	0.80	0.79	0.76	0.75					
Spok-1	$I_{(0)}$	75	78	85	70	86	79					0.86
	$I_{(5)}$	75	80	88	73	90	81					
	$I_{(15)}$	73	77	88	72	91	80					
	$T_{(5)}$	1.00	1.03	1.04	1.04	1.05	1.03	1.35	1.03			
	$T_{(15)}$	0.97	0.99	1.04	1.03	1.06	1.02	1.36	1.04			
Spok-2	$I_{(0)}$	80	90	81	89	78	84					0.91
	$I_{(5)}$	80	93	83	92	80	86					
	$I_{(15)}$	81	91	80	89	76	83					
	$T_{(5)}$	1.00	1.03	1.02	1.03	1.03	1.02	1.35	1.02			
	$T_{(15)}$	1.01	1.01	0.99	1.00	0.97	1.00	1.34	1.02			
Spok-3	$I_{(0)}$	90	75	79	86	72	80					0.87
	$I_{(5)}$	91	78	82	87	74	82					
	$I_{(15)}$	90	74	80	88	73	81					
	$T_{(5)}$	1.01	1.04	1.04	1.01	1.03	1.03	1.35	1.02			
	$T_{(15)}$	1.00	0.99	1.01	1.02	1.01	1.01	1.35	1.03			

$I_{(0)}$ is the light reading after the initial five minute incubation period

$I_{(5)}$ is the light reading five minutes after $I_{(0)}$

$I_{(15)}$ is the light reading fifteen minutes after $I_{(0)}$

$C_{(t)}$, $R_{(t)}$, and $T_{(t)}$ are the changes in light readings from the intial reading in each sample container for the control, reference sediment and test sites. $I_{(t)}/I_{(0)}$

Quality Control Steps:

1. Is control final mean output greater than 72% control initial mean output?

   $I_{(5)}$: $F_{c(mean)}/I_{c(mean)}$=100%

   $I_{(15)}$: $F_{c(mean)}/I_{c(mean)}$=98%

Yes. Control results are acceptable.

2. Does the reference final mean exceed 80% of control final mean?

   $I_{(5)}$: $F_{R(mean)}/F_{C(mean)}$=76%

   $I_{(15)}$: $F_{R(mean)}/F_{C(mean)}$=76%

No. Reference site results are not acceptable to be used in statistical analyses.

3. Is the reference initial mean > 80% of control initial mean?

   $I_{R(mean)}/I_{C(mean)}$=76%

No. Reference initial mean should not be used to calculate change in light readings at $I_{(5)}$ and $I_{(15)}$ for reference site.

4. Are test initial mean values > 80% of control initial mean values?

   Spok-1: $I_{T(mean)}/I_{C(mean)}$=86%, use to calculate change in light readings.

   Spok-2: $I_{T(mean)}/I_{C(mean)}$=91%, use to calculate change in light readings.

   Spok-3: $I_{T(mean)}/I_{C(mean)}$=87%, use to calculate change in light readings.

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APPENDIX B

WATER QUALITY RESULTS

Appendix Table B-1. Microtox Results
303(d) Verification - Sediment Bioassay Study
Washington State Department of Ecology
Water Quality Data

Test Date 21 November 2003

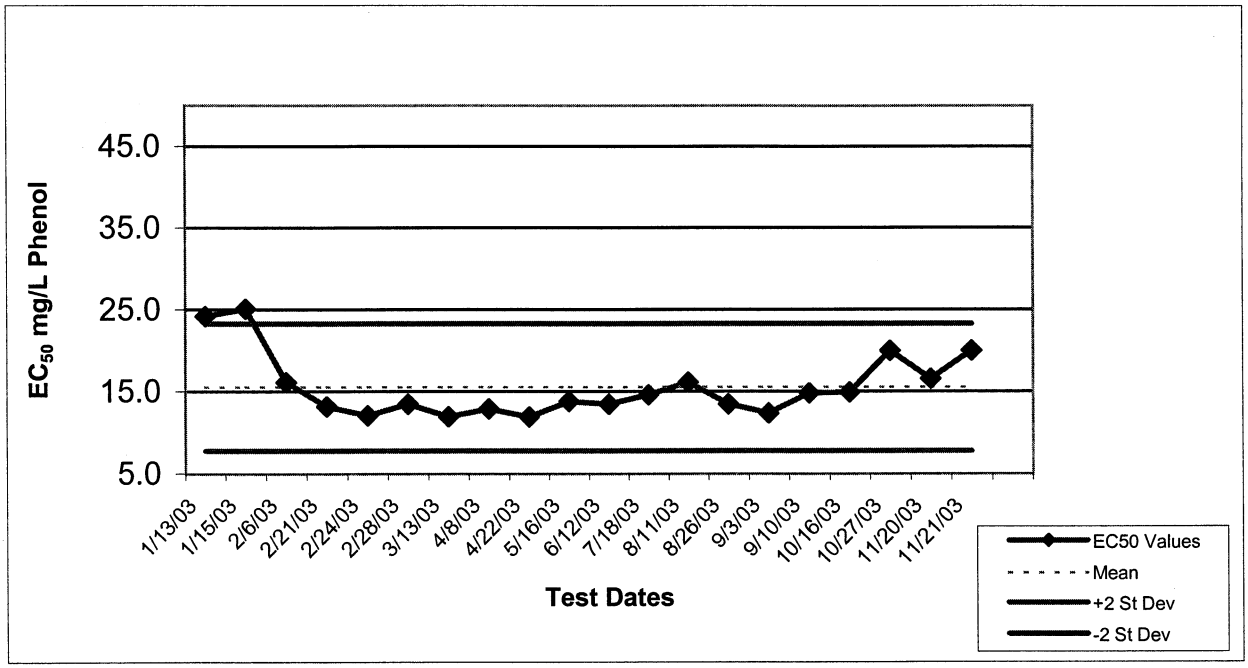
Site	Initial Salinity (ppt)	Final Salinity (ppt)	Initial D.O. (mg/L)	Final D.O. (mg/L)	Initial pH (units)	Final pH (units)	Final Porewater Conc	Total NH_3 (mg/L)
Control	0.0	20.2	7.3	7.3	8.13	8.13	100.0%	NT
Mill-Ref	0.0	20.6	7.1	7.1	7.10	8.13	99%	6.0
Mill-1	0.2	20.8	7.3	7.3	7.25	8.16	99.2%	11.0
Mill-2	0.1	20.5	7.5	7.5	7.67	8.19	100%	5.1
Mill-3	0.2	20.9	7.4	7.4	7.20	8.17	99.2%	5.3
Spok-Ref	0.2	19.8	7.4	7.4	7.05	8.14	98.8%	11.4
Spok-1	0.2	20.9	7.3	7.3	7.74	8.20	99.6%	2.9
Spok-2	0.1	20.7	7.9	7.9	7.92	7.92	100.0%	3.0
Spok-3	0.1	20.9	7.3	7.3	7.75	8.12	99.8%	4.0

APPENDIX C

REFERENCE TOXICANT TEST

Reference Toxicant Control Chart
Microtox 5-Minute Exposure

CV% = 25.0

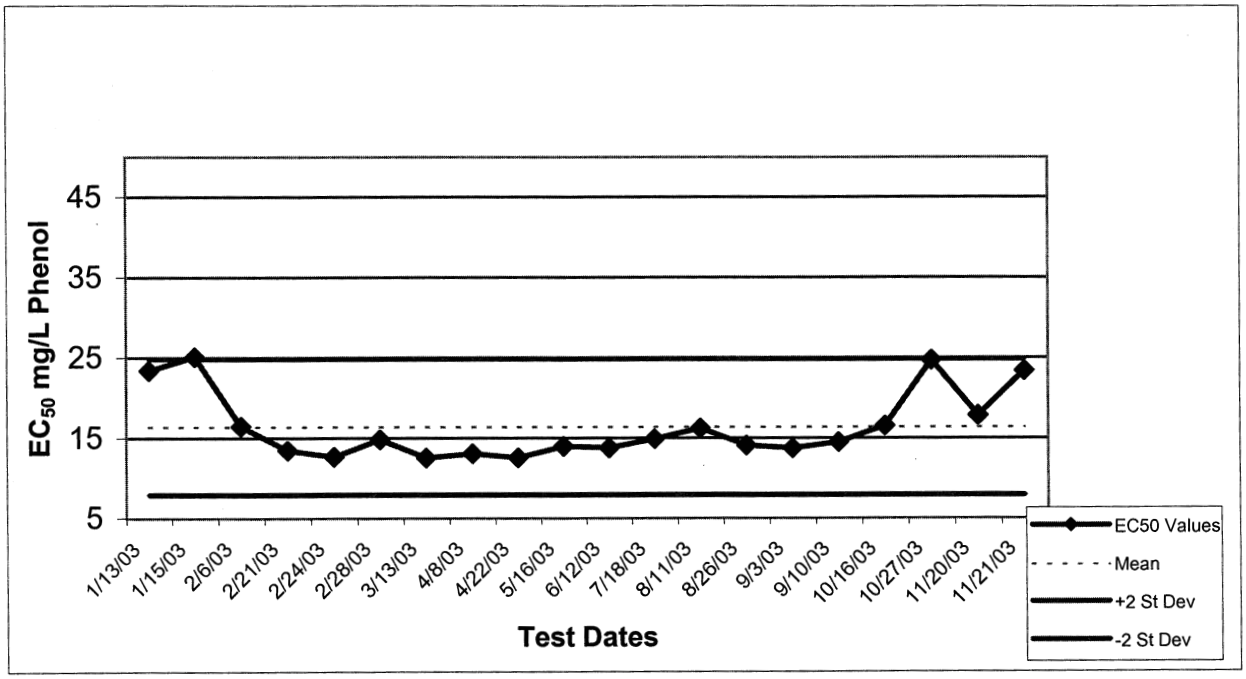


Date	Time	EC50 %	EC50 mg/L Phenol [a]	Mean	StDev	-2 SD	+2 SD
1/13/03	1518	23.7	24.2	15.5	3.88	7.8	23.3
1/15/03	1610	24.6	25.1	15.5	3.87	7.8	23.3
2/6/03	1516	15.8	16.1	15.5	3.87	7.8	23.3
2/21/03	1442	12.9	13.2	15.5	3.87	7.8	23.3
2/24/03	1149	11.8	12.0	15.5	3.87	7.8	23.3
2/28/03	1459	13.2	13.5	15.5	3.87	7.8	23.3
3/13/03	1517	11.7	11.9	15.5	3.87	7.8	23.3
4/8/03	1645	12.6	12.9	15.5	3.87	7.8	23.3
4/22/03	1523	11.7	11.9	15.5	3.87	7.8	23.3
5/16/03	1503	13.5	13.8	15.5	3.87	7.8	23.3
6/12/03	1540	13.2	13.5	15.5	3.87	7.8	23.3
7/18/03	1635	14.3	14.6	15.5	3.87	7.8	23.3
8/11/03	1225	15.8	16.1	15.5	3.87	7.8	23.3
8/26/03	1612	13.2	13.5	15.5	3.87	7.8	23.3
9/3/03	1739	12.1	12.4	15.5	3.87	7.8	23.3
9/10/03	1746	14.5	14.8	15.5	3.87	7.8	23.3
10/16/03	1642	14.6	14.9	15.5	3.87	7.8	23.3
10/27/03	1735	19.6	19.9	15.5	3.87	7.8	23.3
11/20/03	1530	16.2	16.5	15.5	3.87	7.8	23.3
11/21/03	1535	19.6	20.0	15.5	3.87	7.8	23.3

a - Highest concentration of Phenol is 102 mg/L

Control Chart Reference Toxicant Control Chart
Microtox 15-Minute Exposure

CV% = 25.9



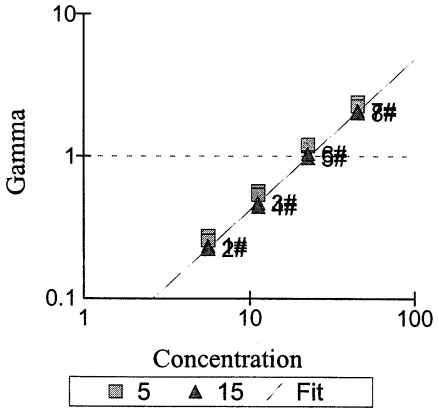
Date	Time	EC50 %	EC50 mg/L Phenol [a]	Mean	StDev	-2 SD	+2 SD
1/13/03	1518	22.9	23.4	16.4	4.23	7.9	24.8
1/15/03	1610	24.6	25.1	16.4	4.23	7.9	24.8
2/6/03	1516	16.1	16.4	16.4	4.23	7.9	24.8
2/21/03	1442	13.2	13.5	16.4	4.23	7.9	24.8
2/24/03	1149	12.4	12.6	16.4	4.23	7.9	24.8
2/28/03	1459	14.5	14.8	16.4	4.23	7.9	24.8
3/13/03	1517	12.3	12.5	16.4	4.23	7.9	24.8
4/8/03	1645	12.8	13.1	16.4	4.23	7.9	24.8
4/22/03	1523	12.3	12.5	16.4	4.23	7.9	24.8
5/16/03	1503	13.7	14.0	16.4	4.23	7.9	24.8
6/12/03	1540	13.5	13.8	16.4	4.23	7.9	24.8
7/18/03	1635	14.6	14.9	16.4	4.23	7.9	24.8
8/11/03	1225	15.9	16.2	16.4	4.23	7.9	24.8
8/26/03	1612	13.8	14.1	16.4	4.23	7.9	24.8
9/3/03	1739	13.5	13.7	16.4	4.23	7.9	24.8
9/10/03	1746	14.2	14.5	16.4	4.23	7.9	24.8
10/16/03	1652	16.2	16.5	16.4	4.23	7.9	24.8
10/27/03	1735	24.2	24.7	16.4	4.23	7.9	24.8
11/20/03	1530	17.5	17.9	16.4	4.23	7.9	24.8
11/21/03	1535	22.9	23.4	16.4	4.23	7.9	24.8

a - Highest concentration of Phenol is 102 mg/L

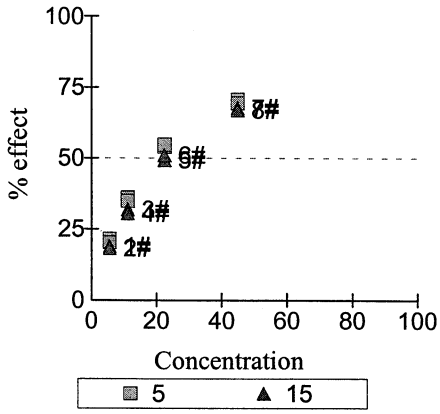
MicrotoxOmni Test Report

Test Protocol: Basic Test
Sample: 102 mg/L phenol
Toxicant: 102mg/L Phenol
Reagent Lot no.: 3B2159 Exp 3/05
Test description: Reference Toxicant
Test name: RT112103VF
Database file: C:\Program Files\MicrotoxOmni\Phenol.mdb

Plot of Gamma vs Concentration



Plot of %Effect vs Concentration



			5 Mins Data:			15 Mins Data:		
Sample	Conc	Io	It	Gamma	% effect	It	Gamma	% effect
Control	0.000	94.75	85.81	0.9056 #		86.92	0.9174 #	
Control	0.000	103.17	89.15	0.8641 #		88.21	0.8550 #	
1	5.625	95.77	66.60	0.2724	21.41%	68.91	0.2316 #	18.80%
2	5.625	97.80	68.99	0.2544 #	20.28%	70.94	0.2217 #	18.15%
3	11.25	93.55	52.94	0.5637 #	36.05%	56.51	0.4670 #	31.84%
4	11.25	67.09	38.69	0.5344 #	34.83%	41.34	0.4382 #	30.47%
5	22.50	95.75	39.01	1.172 #	53.96%	43.08	0.9696 #	49.23%
6	22.50	94.64	37.96	1.206 #	54.67%	41.21	1.035 #	50.86%
7	45.00	98.40	25.62	2.399 #	70.58%	28.32	2.079 #	67.52%
8	45.00	109.91	29.84	2.259	69.32%	32.33	2.013 #	66.81%

- used in calculation; * - invalid data; D - deleted from calcs.
Autocalc has been used.

Calculations on 5 Mins data:
EC50 Concentration:19.56% (95% confidence range: 18.91 to 20.24)
95% Confidence Factor: 1.034
Estimating Equation:LOG C =0.9200 x LOG G +1.291
Coeff. of Determination (R^2):0.9988
Slope: 1.086
Correction Factor: 0.8849

Calculations on 15 Mins data:
EC50 Concentration:22.92% (95% confidence range: 22.12 to 23.74)
95% Confidence Factor: 1.036
Estimating Equation:LOG C =0.9356 x LOG G +1.360
Coeff. of Determination (R^2):0.9983
Slope: 1.067
Correction Factor: 0.8862

APPENDIX D

CHAIN-OF-CUSTODY FORM

WASHINGTON STATE DEPARTMENT OF ECOLOGY

REQUEST FOR LABORATORY SERVICES

SIC NO. _____

Contact: Karen Bergmann	Project: 303(d) Verification	Date: 11/6/03
Laboratory: AMEC Earth & Environmental	Client and Address: WA STATE DEPT. OF ECOLOGY MANCHESTER LABORATORY 7411 BEACH DRIVE EAST PORT ORCHARD WA 98366-8204	❑ Enforcement ❑ Return to Client ❑ Dispose ❑ Return Cooler
Address: 5009 Pacific Hwy E, Suite 2 Fife 98424		

ITEM NO.	SAMPLE NO.	PROJECT NAME AND/OR DESCRIPTION	QUANTITY	UNIT PRICE	TOTAL COST
	~~45-8100-3~~	Analyze eight sediment samples for	8	200	1,600
	~~8108~~ RC	microtox 100% porewater			
	8105-08	toxicity assessment using ~~the~~			
		method WDOE (2003).			
		Deliverables to include a case narrative documenting any sample or analysis anomalies, copies of all raw data, report tables, etc.			
		Please include an invoice			
		Data report due within 15 days after completion of test			
		TOTAL			$ 1,600

Requested By (Your contact if any questions arise): _____ Phone No. _____

CHAIN OF CUSTODY*

Relinquished By:	Received By:	Yr	Mo	Day	Hr	Min
Will White		03	11	12	12	55

*Signatures on this part of the form pertain to the custody of these samples and not to the cost of the analysis.

Invoice will be paid after sample analyses have passed a QA/QC review.

REPORT

of

TEST NO. 702-2

***CHIRONOMUS TENTANS* 20-DAY SEDIMENT TOXICITY TEST OF FRESHWATER SEDIMENTS**

Submitted to

WASHINGTON STATE DEPARTMENT OF ECOLOGY
MANCHESTER LABORATORY
7411 BEACH DRIVE EAST
PORT ORCHARD, WA 98366-8204

Submitted by

NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD
P.O. BOX 1437
NEWPORT, OR 97365

JANUARY 20, 2004

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 702-2

Title: Toxicity of freshwater sediments using a 20-day chironomid, *Chironomus tentans*, sediment bioassay.

Protocol No.: NAS-XXX-CT4c, October 18, 2000. Rev. 1 (Nov. 8, 2003). Based on ASTM 2001 (Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates, E1706-00), Am. Soc. Test. Mat., Phila., PA, and EPA Method 100.1 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-99/064). Testing also complied with Sediment Management Standards (SMS)(Washington Department of Ecology 2003. *Sediment Sampling and Analysis Plan Appendix: Guidance on the Development of Sediment Sampling and Analysis Plans Meeting the Requirements of the Sediment Management Standards (Chapter 173-204 WAC)*).

STUDY MANAGEMENT

Study Sponsor: Washington State Department of Ecology, Manchester Laboratory, 7411 Beach Drive East, Port Orchard, WA 98366-8204

Sponsor's Study Monitor: Ms. Brandee Era-Miller

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365

Test Location: Newport laboratory

Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R.S. Caldwell, PhD, Sr. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; M.S. Redmond, M.S., Aq. Toxicol.; G.C. Hayes, B.S., Sr. Tech.; W.T. Montgomery, A.A., Tech.

Study Schedule:

Test Beginning: 11-18-03, 1730 hrs.

Test Ending: 12-8-03, 1500 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Freshwater sediments. Details follow:

NAS Sample No.	8623F	8624F	8625F	8626F
WDOE Sample No.	458100	458101	458102	458103
WDOE Description	SPOK-1	SPOK-2	SPOK-3	SPOK-REF
Collection Date	11-6-03	11-6-03	11-6-03	11-5-03
Receipt Date	11-13-03	11-13-03	11-13-03	11-13-03

NAS Sample No.	8627F	8628F	8629F	8630F
WDOE Sample No.	458105	458106	458107	458108
WDOE Description	MILL-1	MILL-2	MILL-3	MILL-REF
Collection Date	11-7-03	11-7-03	11-8-03	11-8-03
Receipt Date	11-13-03	11-13-03	11-13-03	11-13-03

Reference Sediment: A single reference sediment, NAS #8626F (458103, SPOK-REF), was specified by the client to be used for statistical comparisons.

Control Sediment: The negative control sediment (NAS #8519F) was collected on 11-12-03 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR. The control sediment was press sieved through a 0.5 mm screen.
Treatments: Homogenized at test set up by mixing using stainless steel implements.
Storage: All test and reference sediments were stored at 4°C in the dark in capped containers until used.

TEST WATER

Source: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Dates of Preparation: 11-16-03, 11-18-03, 11-19-03, 11-20-03 (two batches), 11-23-03, 11-24-03, 11-25-03, 11-28-03 (2 batches), 11-30-03, 12-1-03, 12-3-03, 12-5-03
Water Quality:

pH: 8.3, 8.2, 7.9, 8.0, 8.1, 7.9, 7.6, 8.1, 8.3, 8.1, 8.1, 8.1, 8.2, 8.2 (8.1± 0.2, mean ± SD)
Conductivity: 295, 290, 290, 300, 280, 280, 280, 280, 270, 270, 280, 280, 280, 280 (282 ± 8 µmhos/cm, mean ± SD)
Hardness: 94, 94, 102, 94, 85, 102, 85, 94, 94, 85, 94, 85, 85, 102 (92 ± 7 mg/L as $CaCO_3$, mean ± SD)
Alkalinity: 80, 80, 80, 70, 70, 80, 70, 80, 70, 70, 80, 70, 70, 80 (75 ± 5 mg/L as $CaCO_3$, mean ± SD)

Pretreatment: Aerated ≥24 hr.

TEST ORGANISMS

Species: *Chironomus tentans*, midge.
Age/Size: 1st instar, <24 hrs. old
Source: NAS cultures
Acclimation: Egg cases were placed into moderately hard dilution water when isolated and held at test conditions during development and hatch. Holding conditions averaged: Temperature, 23.5 ± 0.6°C; dissolved oxygen, 7.7 ± 0.2 mg/L; pH, 8.1 ± 0.1; conductivity, 333 ± 12 µmhos/cm; hardness, 94 mg/L as $CaCO_3$; and alkalinity, 80 mg/L as $CaCO_3$. The photoperiod was 16:8, L:D.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a detailed description of the test procedures used in this study.

Test Chambers: 300 ml high-form glass beakers
Test Volumes: 100 ml sediment layer; 175 ml test water.
Replicates/Sample: 8
Organisms/Sample: 80
Water Volume Changes: 2 water volumes per day
Aeration: Initiated in all beakers on day 13 because dissolved oxygen fell below 2.5 mg/L in one replicate.
Feeding: Animals were fed 1.5 ml of TetraFin™ suspension (1.5 ml contains 6 mg dry solids) per beaker daily.
Effects Criteria: 1) survival after 20 days, and 2) average individual biomass (based on ash-free dry weight) after 20 days. Death is defined as no visible movement or response to tactile stimulation. Missing organisms were considered to be dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, conductivity, pH, hardness, alkalinity, sulfide and ammonia-nitrogen were measured in the overlying water of one replicate test container per treatment on days 0 and 20 of the test. Temperature was measured daily, dissolved oxygen and pH three times per week, and conductivity weekly, in the overlying water of one replicate test container per treatment. Hardness and alkalinity were measured using titrimetric methods. Total soluble sulfide and total ammonia-N were measured using Hach test kits based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. The photoperiod was 16:8, L:D.

DATA ANALYSIS METHODS

Survival and individual biomass were calculated for each sample replicate as follows:

percent survival = 100 x (number surviving/initial number tested)
ash-free dry wt. = dry weight of organisms recovered on day 20 – ashed dry weight, in mg
where:
average individual ash-free biomass = (ash-free dry wt.)/number weighed

Means and standard deviations for biological endpoints and for water quality data were computed using Microsoft Excel 2000. Percent survival and average individual ash-free biomass in each test sediment were statistically compared against that in the control and in the reference sediment. An arcsine square root, rank-order, or rankits transformation was performed on percent survival data before analysis. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.05 level of significance. The software used for statistical comparisons was BioStat (Beta v.4.1 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District.

PROTOCOL DEVIATIONS

1. Maximum values for hardness (102 mg/L) and alkalinity (80 mg/L) measured in the moderately hard synthetic dilution water were slightly above the limits set in the protocol of 80-100 mg/L and 60-70, respectively.
2. Afternoon water renewal was not conducted on day zero. This was done to prevent disturbing the larvae before they had had time to settle into the sediment.

REFERENCE TOXICANT TEST

The reference toxicant test is a multi-concentration toxicity test using potassium chloride, to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below.

Test No.: 999-1705
Reference Toxicant and Source: Potassium chloride (Fisher, Lot No. 006829).
Test Date: 11-18-03
Dilution Water Used: Moderately hard synthetic water prepared 11-16-03 from Milli-Q® deionized water.
Result: 96-hr LC50, 2.28 g/L. This result is within the laboratory's control chart warning limits (1.51 to 4.11 g/L).

TEST RESULTS

Observations of water quality in the overlying water throughout the test are summarized in Table 1. A detailed tabulation of the water quality results by sample and test day can be found in Appendix II. The means and standard deviations of percent survival and growth (ash-free dry weight) of midges exposed for 20 days to sediments are summarized in Tables 2 and 3, respectively. Detailed data organized by sample and replicate, and summary statistics for these observations, are given in Appendix II.

Except as noted above, all observations of overlying water quality were within the protocol specified ranges. Sulfides in the overlying water for all samples on days 0 and 20 were <0.02 mg/L. Ammonia-N levels in the overlying water for day 0 and day 10 ranged from <0.1 to 0.9 mg/L.

The test met the acceptability criteria specified in the protocol. Mean control survival was 90.0% (70% required). The 1.22 mg average individual ash-free dry weight of the controls exceeded the test acceptance requirement of ≥0.48 mg ash-free dry weight. The reference sediment 458103 (SPOK-REF) resulted in 76.3% survival. The reference toxicant (positive control) result was within the laboratory's control chart limits

(2.28 g KCl/L; control chart mean ± 2 S.D. = 2.81 ± 1.30). It is concluded, therefore, that the test has developed fully acceptable data for use in making management decisions.

All test sediments, as well as reference sediment 458103 (SPOK-REF), had survival values significantly lower than that in the control. Percent survival in four sediments (458100 [SPOK-1], 458102 [SPOK-3], 458105 [MILL-1], and 458108 [MILL-REF]) was significantly lower than that in reference sediment 458103 (SPOK-REF)(Table 2).

The ash-free biomass of larvae in all test sediments, but not in reference sediment 458103 (SPOK-REF), was significantly lower than that of the control larvae. The ash-free biomass in all test sediments except 458106 (MILL-2) was significantly lower than that in the reference sediment (Table 3).

STUDY APPROVAL

_____

_____

Project Manager/Study Director Date Quality Assurance Unit Date

_____

Laboratory Director Date

Table 1. Summary of water quality conditions during the bioassay of the midge, *Chironomus tentans*, exposed to freshwater sediments.

Water Quality Parameter	Mean ± S.D.	Minimum	Maximum	N
Temperature (°C)	23.1 ± 0.4	22.1	23.9	189
Dissolved oxygen (mg/L)	5.5 ± 1.6	1.7	7.9	99
Conductivity (µmhos/cm)	283 ± 13	260	320	45
pH	7.4 ± 0.3	6.8	8.0	90
Hardness (mg/L as $CaCO_3$)	92 ± 7	85	102	18
Alkalinity (mg/L as $CaCO_3$)	77 ± 6	70	90	18
Sulfides (mg/L)	---	<0.02	<0.02	18
Total ammonia (mg/L)	---	<0.1	0.9	18

Table 2. Survival results of *Chironomus tentans* exposed to freshwater sediments for 20-days.

Sample description	Percent survival (Mean ± SD)	Significantly lower than in control sediment at α = 0.05?	Significantly lower than in reference sediment at α = 0.05?
Control sediment (NAS#8519F)	90.0 ± 10.7	---	---
Reference sediment 458103 SPOK-REF (NAS #8626F)	76.3 ± 16.0	Yes	---
458100 SPOK-1 (NAS #8623F)	55.0 ± 21.4	Yes	Yes
458101 SPOK-2 (NAS #8624F)	67.5 ± 17.5	Yes	No
458102 SPOK-3 (NAS #8625F)	50.0 ± 28.3	Yes	Yes
458105 MILL-1 (NAS #8627F)	57.5 ± 26.0	Yes	Yes
458106 MILL-2 (NAS #8628F)	63.8 ± 13.0	Yes	No
458107 MILL-3 (NAS #8629F)	70.0 ± 20.7	Yes	No
458108 MILL-REF (NAS #8630F)	5.0 ± 7.6	Yes	Yes

Table 3. Growth results of *Chironomus tentans* exposed to freshwater sediments for 20-days.

Sample description	Average Ash-Free Dry wt/Larvae (mg) (Mean ± SD)	Significantly lower than in control sediment at α = 0.05?	Significantly lower than in reference sediment at α = 0.05?
Control sediment (NAS#8519F)	1.22 ± 0.09	---	---
Reference sediment 458103 SPOK-REF (NAS #8626F)	1.11 ± 0.18	No	---
458100 SPOK-1 (NAS #8623F)	0.83 ± 0.19	Yes	Yes
458101 SPOK-2 (NAS #8624F)	0.82 ± 0.13	Yes	Yes
458102 SPOK-3 (NAS #8625F)	0.56 ± 0.33	Yes	Yes
458105 MILL-1 (NAS #8627F)	0.64 ± 0.27	Yes	Yes
458106 MILL-2 (NAS #8628F)	1.00 ± 0.19	Yes	No
458107 MILL-3 (NAS #8629F)	0.81 ± 0.25	Yes	Yes
458108 MILL-REF (NAS #8630F)	0.06 ± 0.04	Yes	Yes

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REPORT

of

TEST NO. 702-1

HYALELLA AZTECA 10-DAY SEDIMENT TOXICITY TEST OF FRESHWATER SEDIMENTS

Submitted to

WASHINGTON STATE DEPARTMENT OF ECOLOGY
MANCHESTER LABORATORY
7411 BEACH DRIVE EAST
PORT ORCHARD, WA 98366-8204

Submitted by

NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD
P.O. BOX 1437
NEWPORT, OR 97365

JANUARY 20, 2004

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 702-1

Title: Toxicity of freshwater sediments using a 10-day amphipod, *Hyalella azteca*, sediment bioassay.

Protocol No.: NAS-XXX-HA4b, April 7, 1998. Rev. 1 (Oct. 27, 2003). Based on ASTM 2001 (Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates, E1706-00), Am. Soc. Test. Mat., Phila., PA, and EPA Method 100.1 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-99/064). Testing also complied with Sediment Management Standards (SMS)(Washington Department of Ecology 2003. *Sediment Sampling and Analysis Plan Appendix: Guidance on the Development of Sediment Sampling and Analysis Plans Meeting the Requirements of the Sediment Management Standards (Chapter 173-204 WAC)*).

STUDY MANAGEMENT

Study Sponsor: Washington State Department of Ecology, Manchester Laboratory, 7411 Beach Drive East, Port Orchard, WA 98366-8204

Sponsor's Study Monitor: Ms. Brandee Era-Miller

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365

Test Location: Newport laboratory

Laboratory's Study Personnel G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R.S. Caldwell, PhD, Sr. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; M.S. Redmond, M.S., Aq. Toxicol.; G.C. Hayes, B.S., Sr. Tech.; W.T. Montgomery, A.A., Tech.

Study Schedule:

Test Beginning: 11-18-03, 1130 hrs.

Test Ending: 11-28-03, 1100 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Freshwater sediments. Details follow:

NAS Sample No.	8623F	8624F	8625F	8626F
WDOE Sample No.	458100	458101	458102	458103
WDOE Description	SPOK-1	SPOK-2	SPOK-3	SPOK-REF
Collection Date	11-6-03	11-6-03	11-6-03	11-5-03
Receipt Date	11-13-03	11-13-03	11-13-03	11-13-03

NAS Sample No.	8627F	8628F	8629F	8630F
WDOE Sample No.	458105	458106	458107	458108
WDOE Description	MILL-1	MILL-2	MILL-3	MILL-REF
Collection Date	11-7-03	11-7-03	11-8-03	11-8-03
Receipt Date	11-13-03	11-13-03	11-13-03	11-13-03

Reference Sediment: A single reference sediment, NAS #8626F (458103, SPOK-REF), was specified by the client to be used for statistical comparisons.

Control Sediment: The negative control sediment (NAS #8519F) was collected on 11-12-03 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR. The control sediment was press sieved through a 0.5 mm screen.
Treatments: Homogenized at test set up by mixing using stainless steel implements.
Storage: All test and reference sediments were stored at 4°C in the dark in capped containers until used.

TEST WATER

Source: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Dates of Preparation: 11-16-03, 11-18-03, 11-19-03, 11-20-03, 11-21-03, 11-23-03, 11-24-03, 11-25-03
Water Quality: pH 8.3, 8.2, 7.9, 8.0, 8.1, 7.9, 7.6, 8.1 (8.0 ± 0.2, mean ± SD)
Conductivity 295, 290, 290, 300, 280, 280, 280, 280 µmhos/cm (287 ± 8, mean ± SD)
Hardness 94, 94, 102, 94, 85, 102, 85, 94 mg/L as $CaCO_3$ (94 ± 6, mean ± SD)
Alkalinity 80, 80, 80, 70, 70, 80, 70, 80 mg/L as $CaCO_3$ (76 ± 5, mean ± SD)
Pretreatment: Aerated ≥24 hr.

TEST ORGANISMS

Species: *Hyalella azteca,* amphipod.
Age/Size: 10-11 days old
Source: Chesapeake Cultures, Hayes, VA; received 11-14-03
Acclimation: Holding conditions prior to testing averaged: temperature, 21.9 ± 2.4 °C; dissolved oxygen, 9.7 ± 3.0 mg/L; pH, 8.3 ± 0.1; conductivity, 328 ± 27 µmhos/cm; hardness, 118 ± 26 mg/L as $CaCO_3$; and alkalinity, 112 ± 43 mg/L as $CaCO_3$. Half of the water was replaced daily with moderately hard water during holding. Animals were fed YTC daily during holding. The photoperiod was 16:8, L:D.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 300 ml high-form glass beakers
Test Volumes: 100 ml sediment layer; 175 ml test water.
Replicates/Treatment: 8
Organisms/Treatment: 80
Water Volume Changes: 2 water volumes per day
Aeration: none.
Feeding: Animals are fed 1.0 ml of YCT suspension per beaker daily.
Effects Criteria: Survival after 10 days. Death is defined as no visible movement or response to tactile stimulation. Missing organisms were considered to be dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, conductivity, pH, hardness, alkalinity, sulfide and ammonia-nitrogen were measured in the overlying water of one replicate test container per treatment on days 0 and 10 of the test. Temperature and dissolved oxygen were measured daily in the overlying water of one replicate test container per treatment. Hardness and alkalinity were measured with titrimetric methods. Total soluble sulfide and total ammonia-N were measured using Hach test kits based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. The photoperiod was 16:8, L:D.

DATA ANALYSIS METHODS

The end point calculations were as follow:

mortality = initial number – number surviving
percent survival = 100 x (number surviving/initial number tested)
percent mortality = 100 x (mortality/initial number tested)

Means and standard deviations for biological endpoints and for water quality data were computed using Microsoft Excel 2000.

Percent survival in each test sediment was statistically compared against that in the control and in the reference sediment. An arcsine square root, rank-order, or rankits transformation was performed on percent survival data before analysis. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.05 level of significance. The software used for statistical comparisons was BioStat (Beta v.4.1 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District.

PROTOCOL DEVIATIONS

1. Maximum values for hardness (102 mg/L) and alkalinity (80 mg/L) measured in the moderately hard synthetic dilution water were slightly above the limits set in the protocol of 80-100 mg/L and 60-70, respectively.
2. Temperature on day 8 dropped below the 22.0-24.0°C specified in the test protocol (minimum 21.3°C).

These deviations are considered to be minor and should have no effect upon the test results.

REFERENCE TOXICANT TEST

The reference toxicant test is a standard multi-concentration toxicity test using cadmium as $CdCl_2 \bullet 2½ H_2O$, to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. Detailed data and summary statistics are given in Appendix III.

Test No.: 999-1704
Reference Toxicant and Source: Cadmium as $CdCl_2 \bullet 2½ H_2O$, Mallinckrodt Lot #TNZ, 1.0 mg/ml stock prepared 4-2-03.
Test Date: 11-18-03.
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 6.67 µg Cd/L. This result is within the laboratory's control chart warning limits (4.68 - 16.6 µg Cd/L).

TEST RESULTS

Observations of water quality in the overlying water throughout the test are summarized in Table 1. A detailed tabulation of the water quality results by sample and test day can be found in Appendix II. The means and standard deviations of percent survival of *Hyalella azteca* exposed for 10 days to sediments are summarized in Table 2. Detailed data organized by sample and replicate, and summary statistics for these observations, are given in Appendix II.

Except as noted above, all water quality observations of overlying water temperature and dissolved oxygen were within the protocol specified ranges. Sulfides in the overlying water for all samples on days 0 and 10 were <0.02 mg/L. Ammonia-N levels in the overlying water for day 0 and day 10 ranged from <0.1 to 0.6 mg/L.

The test met the acceptability criteria specified in the protocol with 98.8% mean control survival (80% required). The reference sediment 458103 (SPOK-REF) resulted in 100.0% survival. The reference toxicant (positive control) result was within the laboratory's control chart limits (6.67 µg Cd/L; control chart mean ± 2 S.D. = 10.7 ± 5.97). It is concluded, therefore, that the test has developed fully acceptable data for use in making management decisions.

No test sediments had survival values significantly lower than that in the control. Percent survival in four sediments (458101 [SPOK-2], 458102 [SPOK-3], 458106 [MILL-2], and 458108 [MILL-REF]) was

significantly lower than that in reference sediment 458103 (SPOK-REF). However, in no case was the difference in percent survival between test and reference sediment greater than 5%.

STUDY APPROVAL

Project Manager/ Study Director	Date	Quality Assurance Unit	Date
Laboratory Director	Date		

Table 1. Summary of water quality conditions during tests of the amphipod, *Hyalella azteca*, exposed to freshwater sediments.

Water Quality Parameter	Mean ± S.D.	Minimum	Maximum	N
Temperature (°C)	22.7 ± 0.5	21.3	23.5	99
Dissolved oxygen (mg/L)	6.0 ± 0.7	4.2	7.4	99
Conductivity (µmhos/cm)	277 ± 13	260	300	18
pH	7.5 ± 0.2	7.2	7.7	18
Hardness (mg/L as $CaCO_3$)	86 ± 8	68	102	18
Alkalinity (mg/L as $CaCO_3$)	71 ± 5	60	80	18
Sulfide (mg/L)	---	<0.02	<0.02	18
Total ammonia (mg/L)	---	<0.1	0.6	18

Table 2. Survival of amphipods, *Hyalella azteca*, in a 10-day toxicity test. Test sediment survival was statistically compared to that in the control and the reference sediments ($p \leq 0.05$).

Sample description	Percent Survival (Mean ± SD)	Significantly lower than in control sediment at $\alpha = 0.05$?	Significantly lower than in reference sediment at $\alpha = 0.05$?
Control sediment (NAS#8519F)	98.8 ± 3.5	---	---
Reference sediment 458103 SPOK-REF (NAS #8626F)	100.0 ± 0.0	No	---
458100 SPOK-1 (NAS #8623F)	98.8 ± 3.5	No	No
458101 SPOK-2 (NAS #8624F)	96.3 ± 5.2	No	Yes
458102 SPOK-3 (NAS #8625F)	96.3 ± 5.2	No	Yes
458105 MILL-1 (NAS #8627F)	97.5 ± 4.6	No	No
458106 MILL-2 (NAS #8628F)	95.0 ± 7.6	No	Yes
458107 MILL-3 (NAS #8629F)	100.0 ± 0.0	No	No*
458108 MILL-REF (NAS #8630F)	96.3 ± 5.2	No	Yes

* Could not be analyzed statistically, however the value is exactly the same as in the reference sediment.

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Appendix E

Sediment Bioassay SQS/CSL Information

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Table E-1. Sediment Quality Standards (SQS) and Cleanup Screening Levels (CSL) Endpoints for Biological Tests (table adapted from SAIC and Avocet Consulting, 2002 and 2003)

Test	QA Control	QA Reference	SQS	CSL
Hyalella azteca 10-day mortality	C ≤ 20%	R ≤ 25%	T – R > 10%	T – R > 25%
Chironomus tentans 20-day mortality	C ≤ 32%	R ≤ 35%	T – R > 15%	T – R > 25%
Chironomus tentans 20-day growth	CF ≥ 0.48 mg/ind	RF/CF ≥ 0.8	T/R < 0.75	T/R < 0.6
Microtox decrease in Luminescence	CF/CI ≥ 0.72, CF/CI ≤ 1.1	RF/CF ≥ 0.8, RF/CF ≤ 1.1	T/R < 0.85	T/R < 0.75

C = Control
CI = Control Initial
CF = Control Final
R = Reference
RF = Reference Final
T = Test Sample

Table E-2. Sediment Quality Standards (SQS) and Cleanup Screening Levels (CSL) Exceedances for Mill Creek and Spokane River Sediment Bioassays

Site name	10-day *Hyalella azteca* (% Survival ± SD)	20-day *Chironomus tentans* (% Survival ± SD)	20-day *Chironomus tentans* growth (mg-dry weight ± SD)	5-minute Microtox (percent light output ± SD)	15-minute Microtox (percent light output ± SD)	5-minute Microtox (percent light output ± SD)	15-minute Microtox (percent light output ± SD)
Negative Control	98.8 ± 3.5	90.0 ± 10.7	1.22 ± 0.09	100.5 ± 1.9	98 ± 2.4	97.0 ± 0.5	90.6 ± 0.6
Reference: Buffalo Lake	100.0 ± 0.0	76.3 ± 16.0*	1.11 ± 0.18	76.1 ± 6.5**	74.6 ± 6.5**	61.3 ± 11.7**	57.2 ± 11.4**
Spokane River-1	98.8 ± 3.5	55.0 ± 21.4**	0.83 ± 0.19**	103.0 ± 1.9	101.7 ± 3.5	--	--
Spokane River-2	96.3 ± 5.2	67.5 ± 17.5*	0.82 ± 0.13**	102.3 ± 1.4	99.7 ± 1.4	--	--
Spokane River-3	96.3 ± 5.2	50.0 ± 28.3**	0.56 ± 0.33**	102.6 ± 1.4	100.7 ± 1.4	--	--
Upper Mill Creek	96.3 ± 5.2	5.0 ± 7.6**	0.06 ± 0.04**	--	--	73.7 ± 12.3*	68.0 ± 11.9*
Mill Creek-1	97.5 ± 4.6	57.5 ± 26.0**	0.64 ± 0.27**	--	--	97.0 ± 0.8	88.2 ± 2.1
Mill Creek-2	95.0 ± 7.6	63.8 ± 13.0**	1.00 ± 0.19	--	--	97.1 ± 1.2	89.4 ± 0.7
Mill Creek-3	100.0 ± 0.0	70.0 ± 20.7 *	0.81 ± 0.25**	--	--	97.5 ± 1.5	90.4 ± 1.7

Boxed values indicate statistical significance ($p<0.05$) compared to control sample

* Values exceed SQS

** Values exceed CSL

-- = Not applicable, Microtox tests were run in two batches