QUALITY ASSURANCE PROJECT PLAN ADDENDUM 2

Enhanced Natural Recovery/Activated Carbon Pilot Study
Lower Duwamish Waterway
Laboratory Bioaccumulation Study

Lower Duwamish Waterway Group

Port of Seattle / City of Seattle / King County / The Boeing Company

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FINAL

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QUALITY ASSURANCE PROJECT PLAN ADDENDUM 2
LABORATORY BIOACCUMULATION STUDY

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TABLE OF CONTENTS

Page

ABBREVIATIONS AND ACRONYMS v

1.0 PROJECT DESCRIPTION AND OBJECTIVES 1
- 1.1 PLOT LOCATION 2
- 1.2 DATA QUALITY OBJECTIVES 2
  - 1.2.1 Pilot Study DQO-3: Assess Changes in Bioavailability in ENR+AC Compared to ENR Alone 2
- 1.3 PROJECT SCHEDULE 3
- 1.4 QAPP ORGANIZATION 3

2.0 PROJECT ORGANIZATION AND RESPONSIBILITY 4
- 2.1 MANAGEMENT RESPONSIBILITIES 4
  - 2.1.1 Project Management 4
- 2.2 QUALITY ASSURANCE RESPONSIBILITIES 5
- 2.3 FIELD WORK RESPONSIBILITIES 5
- 2.4 LABORATORY RESPONSIBILITIES 5
  - 2.4.1 Bioaccumulation Testing Laboratory 5
  - 2.4.2 SPME Preparation Laboratory 5
  - 2.4.3 Analytical Testing Laboratories 6
- 2.5 SPECIAL TRAINING AND CERTIFICATION 6
- 2.6 DOCUMENTS AND RECORDS 6

3.0 DATA GENERATION AND ACQUISITION 6
- 3.1 OVERVIEW OF THE LABORATORY BIOACCUMULATION STUDY 6
  - 3.1.1 Sampling Event to Obtain Sediment for the Study 6
  - 3.1.2 Laboratory Sediment Core Configuration 9
  - 3.1.3 Laboratory Bioaccumulation Test Procedures 11
  - 3.1.4 Laboratory Porewater Sampling 14
  - 3.1.5 Laboratory and Field Decontamination Procedures 15
- 3.2 ANALYTICAL METHODS 16
  - 3.2.1 Bioaccumulation Tests 16
  - 3.2.2 Tissues Analysis 16
  - 3.2.3 SPME Porewater Sampler Extract Processing and PCB Congener Analysis 17
  - 3.2.4 Quality Assurance Criteria 17
- 3.3 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES 17
  - 3.3.1 QC for Collection of Laboratory and SPME Samples 18
  - 3.3.2 Sample Delivery Group 19
  - 3.3.3 Laboratory QA/QC Criteria 19
- 3.4 FIELD INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE 21
- 3.5 LABORATORY INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY 21
- 3.6 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES 21
- 3.7 DATA REDUCTION 21
- 3.8 DATA AND RECORDS MANAGEMENT 21
  - 3.8.1 EcoAnalysts Laboratory for Bioaccumulation Test Records 21

TABLE OF CONTENTS

Page

4.0 SAMPLE HANDLING AND CUSTODY DOCUMENTATION 22
4.1 SAMPLE NOMENCLATURE 22
4.2 SAMPLE CHAIN-OF-CUSTODY 23
4.3 SAMPLE PRESERVATION, HANDLING, AND TRANSPORT 23
4.3.1 Tissue Samples 23
4.3.2 SPME Extracts for PCB Analysis 24
4.4 SAMPLE RECEIPT 24
5.0 ASSESSMENT AND OVERSIGHT 24
6.0 REPORTING AND RECORD RETENTION 24
6.1 DATA SUBMITTALS AND MONITORING REPORTS 24
6.2 RECORD MAINTENANCE AND STORAGE 25
7.0 REFERENCES 25

TABLES

Table 3.1 Location-Specific Information for Year 3
Table 3.2 Analytical Parameters, Methods, Laboratories, Sample Containers, and Sample Preservation
Table 3.3 Methods and Acceptable Quality Assurance Goals for Biological Testing
Table 3.4 Methods and Acceptable Quality Assurance Criteria for Chemistry in Tissue Samples
Table 3.5 Methods and Acceptable Quality Assurance Criteria for PCB Congeners in Tissue
Table 3.6 Laboratory QA/QC Requirements
Table 4.1 Tissue and SPME Porewater Sample ID Components

FIGURES

Figure 3.1 Subtidal Plot—Sample Composite Grid
Figure 3.2 Schematic of Laboratory Test Chamber Using Sediment Core
Figure 3.3 Schematic of an Array of Cores in the Water Bath and an Example of a Test Core

ATTACHMENT

Attachment A Laboratory Forms

ABBREVIATIONS AND ACRONYMS

AC	activated carbon
AOC	Administrative Order on Consent for Remedial Investigation/Feasibility Study
ASTM	American Society for Testing and Materials
BTL	bioaccumulation testing lead
cm	centimeter
°C	degrees Celsius
DOT	U.S. Department of Transportation
DMMP	Dredged Material Management Program
DQO	data quality objective
Ecology	Washington State Department of Ecology
EDD	electronic data deliverable
ENR	enhanced natural recovery
ENR+AC	enhanced natural recovery amended with activated carbon
EPA	U.S. Environmental Protection Agency
HRGC/HRMS	high resolution gas chromatography with high resolution mass spectroscopy
LDW	Lower Duwamish Waterway
LDWG	Lower Duwamish Waterway Group
Order Amendment	Second Amendment (July 2014) to the Administrative Order on Consent for Remedial Investigation/Feasibility Study
PCB	polychlorinated biphenyl
PDMS	polydimethylsiloxane
ppb	parts per billion
PRC	performance reference compound
QA	quality assurance
QAO	quality assurance officer
QAG	quality assurance goal
QAPP	quality assurance project plan
QC	quality control
SDG	sample delivery group
SOP	standard operating procedure
SPME	solid-phase microextraction
SRM	standard reference material

QUALITY ASSURANCE PROJECT PLAN ADDENDUM 2
Enhanced Natural Recovery/Activated Carbon Pilot Study
Lower Duwamish Waterway
Laboratory Bioaccumulation Study

1.0 PROJECT DESCRIPTION AND OBJECTIVES

As described in the *Quality Assurance Project Plan, Enhanced Natural Recovery/Activated Carbon Pilot Study, Lower Duwamish Waterway* (Pilot Study QAPP), the Lower Duwamish Waterway Group (LDWG) will conduct a pilot study to evaluate the potential effectiveness of using an innovative technology designed to reduce the bioavailability of contaminants in Lower Duwamish Waterway (LDW) sediments. The pilot study is a field study to determine whether enhanced natural recovery (ENR) amended with activated carbon (AC) can be successfully used to decrease contaminant bioavailability compared to ENR without AC. This pilot study was specified under the Second Amendment (July 2014) (Order Amendment; USEPA and Ecology 2014) to the Administrative Order on Consent (AOC) for Remedial Investigation/Feasibility Study (for the Lower Duwamish Waterway, CERCLA Docket No. 10-2001-0055, issued on December 20, 2000 (USEPA and Ecology 2000). Sampling and analytical details of the pilot study are presented in the Pilot Study QAPP (AMEC et al. 2016).

The primary measurement that will be used to assess differences in polychlorinated biphenyl (PCB) bioavailability between the ENR+AC and ENR treatments in the pilot study will be porewater concentrations of PCBs, as measured by solid-phase microextraction (SPME) fibers. This is detailed in the Pilot Study QAPP. As an additional part of the Pilot Study, LDWG will conduct a laboratory bioaccumulation study, which is expected to provide additional support to this line of evidence. The goal of the laboratory study is to determine whether there is a difference in PCB bioavailability due to the addition of AC, as measured by concentrations of PCBs in organisms (i.e., a difference between the ENR and ENR+AC treatments).

This QAPP Addendum serves as an addendum to the Pilot Study QAPP and describes the laboratory bioaccumulation study design, the data quality objectives (DQOs), and the methods for the measurement tools used in the study. Except where noted in this document, methods and quality assurance (QA) procedures are consistent with the Pilot Study QAPP and the reader is referred to the appropriate section of the Pilot Study QAPP. Modifications to this QAPP Addendum may be proposed by LDWG, U.S. Environmental Protection Agency (EPA), or Washington State Department of Ecology (Ecology) after evaluation of data from the Year 1, 2 or 3 sampling events. Modifications would be discussed between all these parties before implementation.

1.1 PLOT LOCATION

The laboratory bioaccumulation study will be conducted with sediment cores collected from the ENR and ENR/AC subplots of the subtidal plot for the Pilot Study. Details on the subtidal plot can be found in Section 1.1 of the QAPP.

1.2 DATA QUALITY OBJECTIVES

This section presents the data quality objectives (DQOs) for the laboratory bioaccumulation study. The DQO process defines criteria that will be used to establish the final data collection design (USEPA 2006). Based on the study goals listed in Section 1.0 of the Pilot Study QAPP, five DQOs were developed to support the selection of sampling and analysis methods and an overall study design for the Pilot Study. The laboratory bioaccumulation study provides supplemental support to DQO-3 of the pilot study: "Assess changes in bioavailability in ENR+AC compared to ENR alone."

The following section presents the overall study design for the laboratory bioaccumulation study, as well as the data types and the analytical approaches that will address the DQO. Specific performance goals, referred to as Data Quality Indicators, for the individual analytical methods are discussed in Section 3.0 after the methods have been introduced.

1.2.1 Pilot Study DQO-3: Assess Changes in Bioavailability in ENR+AC Compared to ENR Alone

The third DQO of the pilot study assesses the potential changes in PCB bioavailability in ENR+AC compared to ENR alone. Under the AOC, changes in bioavailability will be based on changes in porewater PCB concentrations as measured by passive samplers (SPME). The goal of the laboratory bioaccumulation study will provide an additional line of evidence and evaluate the potential changes in PCB bioavailability in ENR+AC compared to ENR alone. Changes in bioavailability will be evaluated based on measurements of PCBs in the tissues of benthic organisms exposed in the laboratory to sediments collected from the subtidal ENR and ENR+AC subplots in Year 3 of the Pilot Study.

Sediment cores will be collected from the top 18 inches of sediment at 18 locations in each of the subtidal subplots. Intact cores will be transferred to the laboratory in a manner that preserves sediment stratigraphy and associated porewater. At the laboratory, marine clams and polychaete worms will be placed into each of the sediment cores and will be exposed to test sediments for 28 days. During the laboratory exposures, porewater PCB concentrations in the sediment cores will be measured using SPME fibers deployed in the top 10 centimeter (cm) of the sediment surface in each of the sediment cores.

The 28-day test duration was selected because longer test duration increases the risk of test species morality as natural carbon sources (nutriment for the invertebrates) in the sediment are depleted. Furthermore, longer duration tests (e.g., the Dredged Material Management Program [DMMP] 45-day test) are conducted with "sediment renewal" at 28-days to "renew" the organic carbon/food for the test organisms. Sediment renewal is not appropriate or possible for this experiment because unacceptable disturbance to the core would occur. Also, the addition of food to the cores would confound evaluation of study results due to potential effects of introduced carbon on PCB availability.

Although steady state conditions may not be achieved in 28-days, steady state is not required to perform the test effectively. 28 days is a standard time frame to evaluate bioaccumulation and will enable evaluation of differences between ENR and ENR+AC, even if steady state is not achieved. In other studies using activated carbon, 28-day bioaccumulation tests have provided robust data to illustrate reductions in PCB bioavailability to invertebrates.

At the end of the 28-day exposures, tissue and porewater PCB concentrations in the sediment from the ENR and ENR+AC subplots will be compared to evaluate the effects on PCB bioavailability due to the addition of AC. If the addition of AC causes the PCBs to remain in the sediment matrix (e.g., adhered to AC), then the amount of PCBs accumulated in the tissues and dissolved in porewater will be less.

1.3 PROJECT SCHEDULE

The laboratory bioaccumulation study will be conducted in conjunction with the Year 3 monitoring event of the Pilot Study (approximately three years after placement of the ENR/AC layers). The cores collected for the laboratory bioaccumulation study will be obtained during the SPME field deployments planned for the Year 3 Pilot Study.

1.4 QAPP ORGANIZATION

This QAPP is organized into the following sections:

- Section 1 – Project Description and Objectives
- Section 2 – Project Organization and Responsibility
- Section 3 – Data Generation and Acquisition
- Section 4 – Sampling Handling and Custody Documentation
- Section 5 – Assessment and Oversight
- Section 6 – Reporting and Record Retention
- Section 7 – References

Representative laboratory forms specific to the laboratory bioaccumulation study are included as Attachment A.

2.0 PROJECT ORGANIZATION AND RESPONSIBILITY

This section describes the overall management of the project, including key personnel, project description, problem definition and background, quality objectives and criteria, special training and certification requirements, and documents and record keeping.

2.1 MANAGEMENT RESPONSIBILITIES

The laboratory bioaccumulation study will be conducted as a component of the Pilot Study. With the exceptions noted below, the project organization and management structure is presented in Section 2.0 of the Pilot Study QAPP.

2.1.1 Project Management

Ms. Meg Pinza of EcoAnalysts, Inc. will serve as the bioaccumulation testing lead (BTL), responsible for the overall design and implementation of the bioaccumulation test exposures. Ms. Pinza is an expert at conducting both laboratory and field exposures for the measurement of toxicity and bioaccumulation. The bioaccumulation testing team reports to the BTL. The BTL reports to the Monitoring Lead (Dr. Victor Magar of Ramboll Environ) who reports to the Program Manager (Mr. Cliff Whitmus). Any out-of-compliance events with the potential to affect data quality or project objectives are reported to the Quality Assurance Officer (Dr. Teri Floyd), as well as the Monitoring Lead and the Program Manager.

Ms. Pinza can be reached as follows:

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PO Box 216
4729 NE View Drive
Port Gamble, WA 98364
Tel: 360.930.4510
MPinza@ecoanalysts.com

Dr. Jason Conder of Geosyntec will serve as the bioaccumulation testing co-lead. Dr. Conder is an expert in the use of SPME sampling techniques for in situ porewater analyses. This expertise includes preparation of the fibers in the laboratory, the addition of special internal standards, deployment and retrieval of the fibers in the laboratory cores, extraction of the fibers before analysis, and interpretation of the results. Dr. Conder will work closely with the Quality Assurance

Officer during the preparation and extraction steps and then will transfer custody of the extracts to the BTL for transportation to the analytical laboratory. During the deployment and retrieval of the fibers in the laboratory exposures, he will work closely with the BTL. Dr. Conder will report to the BTL and Monitoring Lead.

Dr. Conder can be reached as follows:

Jason Conder, PhD
Geosyntec Consultants
2100 Main Street, Suite 150
Huntington Beach, CA 92648
Tel: 714.465.1226
JConder@Geosyntec.com

2.2 QUALITY ASSURANCE RESPONSIBILITIES

Dr. Teri Floyd of Floyd|Snider will serve as the quality assurance officer (QAO) for the monitoring program. The roles and responsibilities for the QAO are stated in the Pilot Study QAPP.

2.3 FIELD WORK RESPONSIBILITIES

The field work and sample collection roles are identified in Section 2.3 of the Pilot Study QAPP. Field work and sample collections activities will be under the direction of Rob Gilmour of Amec Foster Wheeler.

2.4 LABORATORY RESPONSIBILITIES

Dr. Teri Floyd of Floyd|Snider will serve as the overall laboratory coordinator for the monitoring program. Each of the laboratories utilized will adhere to the methods, documentation, reporting, and auditing requirements listed in Section 2.5 of the Pilot Study QAPP.

2.4.1 Bioaccumulation Testing Laboratory

Ms. Meg Pinza will manage the bioaccumulation testing to be performed in EcoAnalysts' laboratory in Port Gamble, Washington.

2.4.2 SPME Preparation Laboratory

Dr. Jason Conder will oversee the preparation of the SPME fibers before deployment and the extraction of the fibers after deployment as detailed in the Pilot Study QAPP.

2.4.3 Analytical Testing Laboratories

All analyses for the laboratory bioaccumulation study will be conducted at Frontier Analytical Laboratory in El Dorado Hills, California. Contact information is presented in the Pilot Study QAPP.

2.5 SPECIAL TRAINING AND CERTIFICATION

The Superfund Amendments and Reauthorization Act of 1986 required the Secretary of Labor to issue regulations providing health and safety standards and guidelines for workers engaged in hazardous waste operations. The federal regulation requires training to provide employees with the knowledge and skills enabling them to perform their jobs safely and with minimum risk to their personal health (Code of Federal Regulations, Title 29, Section 1910.120 [29 CFR 1910.120]). All sampling personnel will have completed the 40-hour HAZWOPER training course and 8-hour refresher courses, as necessary, to satisfy the requirements of the Occupational Safety and Health Administration regulations.

2.6 DOCUMENTS AND RECORDS

Documents and records requirements will follow those presented in Section 2.7 of the Pilot Study QAPP.

3.0 DATA GENERATION AND ACQUISITION

This section describes the collection and handling of porewater, sediment, and biological samples for analysis. Elements include sampling events, sampling design, station location, sampling and analysis methods, laboratory experimental design, QA/QC, and data custody and management.

3.1 OVERVIEW OF THE LABORATORY BIOACCUMULATION STUDY

This section describes the methods for the laboratory bioaccumulation study, including the sampling event to obtain sediment and the design and methods of the laboratory study.

3.1.1 Sampling Event to Obtain Sediment for the Study

There will be one field sampling event in support of the laboratory bioaccumulation study. This event will provide sediment cores from the subtidal ENR and ENR+AC subplots, as detailed below. The sampling event will occur during the final monitoring event of the Pilot Study in 2020, three years after placement of the ENR/AC layers (Year 3). Cores for the laboratory bioaccumulation study will be collected during the SPME field deployments expected to occur between March and May of 2020.

The study design for the laboratory bioaccumulation study will be similar to that of the sediment core collection for the Pilot Study. A full description of the study design for sediment core

collection is found in the Pilot Study QAPP. As with the sediment samples collected during the pilot study, each subtidal subplot sampled for the laboratory bioaccumulation study will be ultimately represented by three composite samples of tissues and SPME fibers exposed to sediment cores collected from 18 locations (6 locations per composite sample, 3 composite samples per subplot). The compositing approach is explained in detail in Sections 3.1.3 below.

The 36 cores from two subplots that will be used for the laboratory bioaccumulation study will be obtained from the two subtidal subplots at the same general location from which porewater and sediment samples will be collected as part of the Year 3 Pilot Study, as shown in Figure 3.1 and Table 3.1 of this QAPP Addendum. Consistent with the Pilot Study QAPP, six of the locations within each subplot have been assigned the letter A, six the letter B, and six the letter C. The sampling event will result in six "A" cores, six "B" cores, and six "C" cores from each of the two subtidal subplots.

3.1.1.1 Core Location Positioning, Collection, and Logging

A total of 18 locations have been defined in each subplot. Methods used for location positioning during the core collection will follow those methods presented in Section 3.2.2 of the Pilot Study QAPP.

Sediment core samples will be collected from the subtidal plots by divers using a pre-cleaned 6-inch-diameter, 24-inch-long cellulose acetate butyrate or Lexan® core liner. Before deployment, the core liners will be decontaminated with warm soapy water using laboratory-grade detergent (e.g., Alconox), triple-rinsed with deionized water, and then sealed to prevent contamination. The target depth interval for core collection will be the uppermost 18 inches (45 cm) of sediment. The core tubes will be marked on the top with brightly colored duct tape or electrical tape. The core liner will also be marked at 18 inches to indicate the depth of insertion.

To collect the sediment core, a core liner will be unsealed and then inserted directly into the sediment surface and gently pushed down into the sediment until the mark is flush with the sediment surface. This sampling depth allows exposure of the test organisms to surface deposits, the ENR/AC layers, and underlying sediment, as well as preserving the porewater within the ENR and underlying sediment layer.

The core liner will not be tilted back and forth into the sediment, although gently rotating the core liner into the sediment while maintaining the core liner in a perpendicular orientation is acceptable. A rod inserted into a hole in the top of the core may be used to assist in rotating the corer; however, they should be easily sealed with the core cap prior to extraction from the sediment. If the core liner cannot penetrate the sediment, the diver may move the location slightly until the target penetration can be reached. If the target penetration cannot be reached after several tries, a

new location will be selected using the procedures in Section 3.2.2 of the Pilot Study QAPP. Once the core liner has been inserted to the target depth, the diver will retrieve the core by first placing the cap on the top of the core liner and then pulling the core liner out of the sediment and immediately capping the bottom of the liner, preventing the release of sediment from the bottom of the core liner. A hand may be placed on the bottom of the core to prevent sediment from being released until the bottom of the core has been capped. In order to keep the sediment stratigraphy and preserve porewater, the diver must ensure that the bottom cap is well seated on the bottom of the core and must keep the core upright after collection and while bringing it to the surface of the water.

Once the core has been brought to the water surface, the on-board crew will inspect the retrieved core length to ensure that the core fully penetrated the ENR layer and into the underlying native sediment and that the upper 18-inch layer is intact. If the underlying native sediment is not present (based on visual observation of the end of the core tube), is not sufficient to block porewater movement out of the bottom of the core, or the core shows significant disturbance during sampling, the core will be recollected in an adjacent location using a new core tube. Once the core has been accepted, the core caps at both ends will then be sealed with electrical/duct tape. The core sample will be labeled with the sample ID (see Section 4.1.1 for naming protocols), date and time, and an arrow pointing toward the top of the core. Intact sediment cores will be stored upright in an ice-filled container (e.g., a cooler) or refrigerator (4 degrees Celsius [C]) during transport to the testing laboratory. Prior to laboratory testing, all cores will be stored in the dark at 4°C (±2°C) oriented vertically. Core handling and holding time requirements for the test sediments are summarized in Table 3.2.

The core will be appropriately sealed both at the top and bottom with a secure end cap and taped to prevent leakage. If water is leaking from the bottom while the core is in transit or storage, the water loss should be evaluated. If water loss is significant such that it indicates a loss of core integrity, the core may be rejected for use in the bioaccumulation study.

The EPA mandates the management of field-generated waste to ensure the protection of the environment and of human health. Field-generated waste will be managed according to the same procedures as provided for sediment core collection in the Pilot Study QAPP (AMEC et al. 2016).

Form: The field technician will complete a surface sediment core sample collection form (Pilot Study QAPP Attachment A) for each core collected. Photographs will be recorded on the photograph log form and cross-referenced to the surface sediment core sample collection form by station location.

3.1.2 Laboratory Sediment Core Configuration

The laboratory bioaccumulation tests will be designed to maintain in-situ sediment and porewater conditions as closely as possible and to provide a proper environment for test organisms. In order to minimize disturbance of the sediment stratigraphy and porewater conditions, organism exposures will be conducted in the 36 intact sediment cores collected from the subtidal plots. Sediment cores will be placed vertically on large, flow-through seawater baths at the EcoAnalysts testing laboratory in Port Gamble, Washington. The water baths maintain test temperatures and allow the test chambers to be easily handled and observed during the testing period.

Approximately 24 hours prior to test initiation, each sediment core will be fitted with a screened port located at the top of the core tube approximately 4 inches above the sediment surface (Figures 3.2

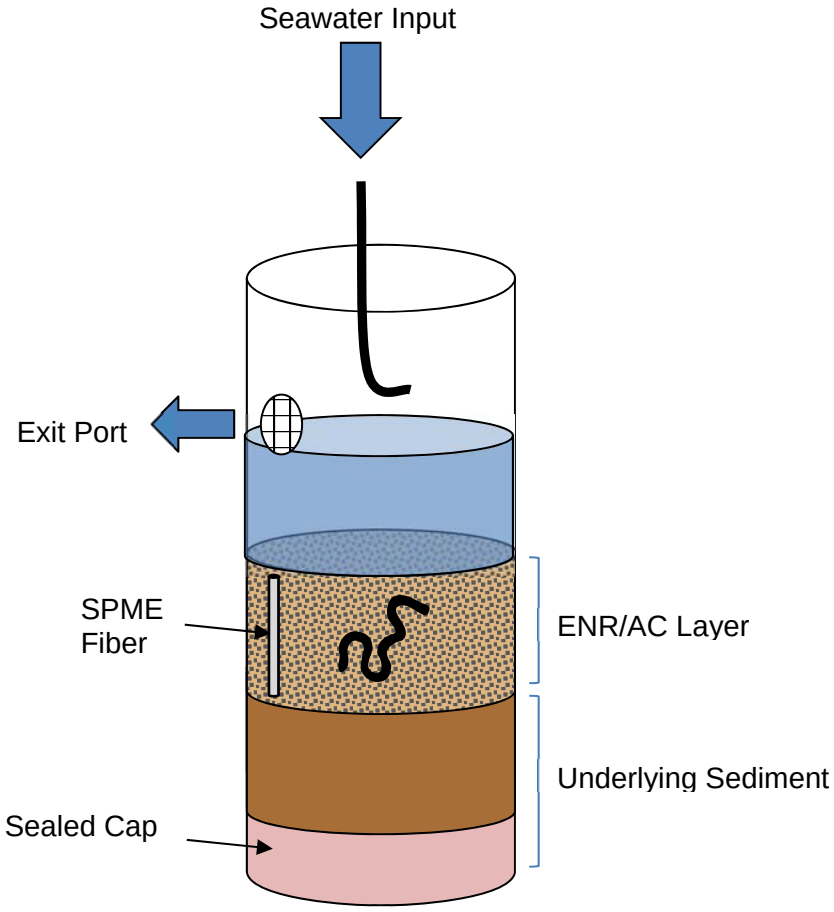

Figure 3.2. Schematic of Laboratory Test Chamber Using Sediment Core
(note: photo is for illustrative purposes only and is not to the size and scale proposed for this experiment).

and 3.3). The screened exit ports allow for overlying-water renewal with minimal disturbance of the sediment-water interface and prevent the potential escape of test organisms. The bottom of the core tube will remain sealed throughout the bioaccumulation exposures. The sediment cores will then be placed upright in pre-assigned random positions within temperature controlled water baths at the test temperature (15°C ± 2°C). The 24-inch-long core tubes will be immersed in the water bath such that the water bath level is 8 to 10 inches below the top of the core tube. Water leaves the exit port from the core and drains into the water bath. The bath level is controlled by a standpipe at one end of the bath, and remains constant regardless of water flow into the test chambers. The water bath consists of seawater of the same temperature as the test chambers. Water from the water bath will not intrude upon the contents of the core tube as the bottom of the tube remains sealed throughout the experiment and the bath's water level is kept below the top of the core and water exchange openings.

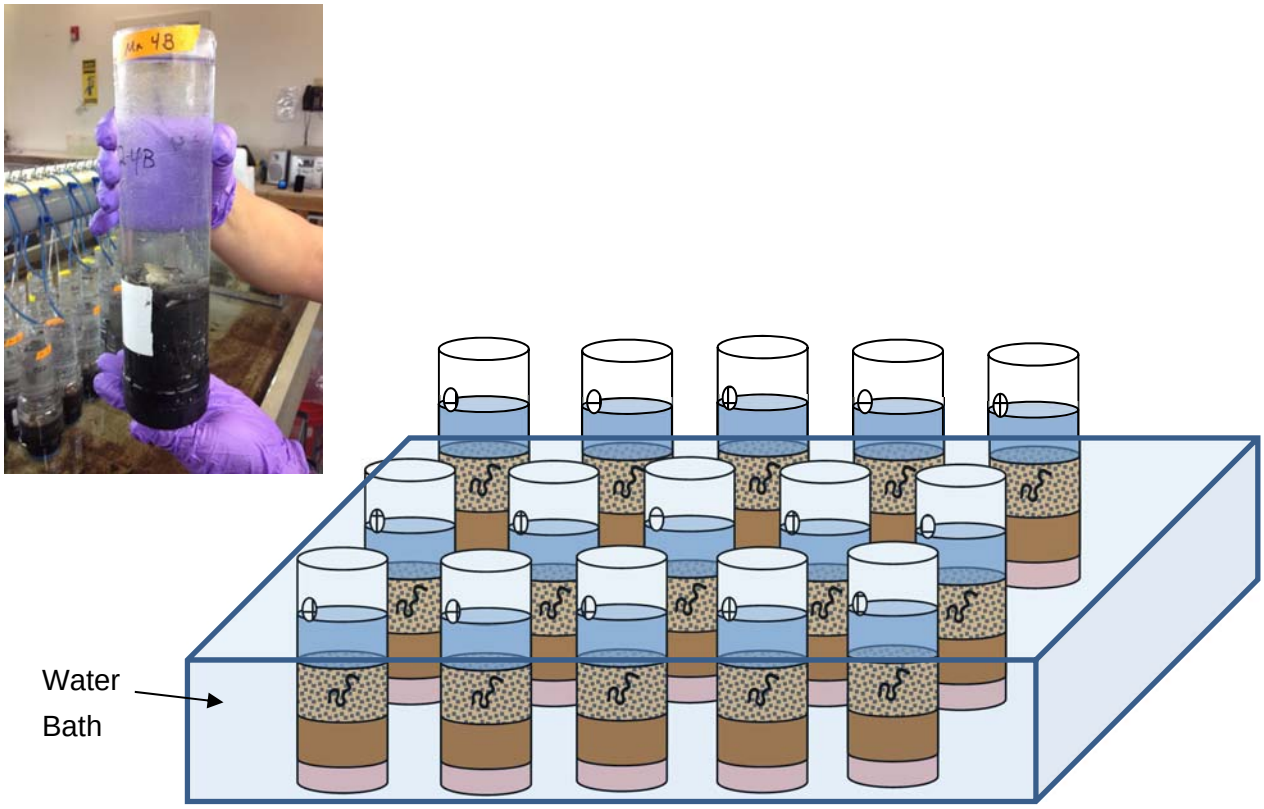

Figure 3.3. Schematic of an Array of Cores in the Water Bath and an Example of a Test Core (note: photo is for illustrative purposes only and is not to the size and scale proposed for this experiment)**.**

After the cores are seated in the water bath, the top core cap of each core tube will be removed. A water supply line will be suspended above each core tube such that each core will be provided with seawater obtained from the EcoAnalysts laboratory seawater system, which is continuously pumped from north Hood Canal, Port Gamble, Washington. This seawater source is routinely used for toxicity and bioaccumulation tests and has been shown to not have PCB Aroclor concentrations at or above 0.01 parts per billion (ppb). A flow of seawater will be provided to each core to allow a "slow-flow" continuous flow-through seawater exchange of the overlying water in each core tube at a rate simulating two tidal exchanges per day. Trickle flow aeration will be provided to each core to maintain the dissolved oxygen concentrations throughout the exposure.

Control sediment exposures will be tested concurrent to the test sediment exposures. The controls will provide an indication of test organism health during the 28-day exposures. The controls will be comprised of six 6-inch-diameter core tubes containing approximately 18 inches of clean sediment. The sediment that will be used in the control chambers is collected at the same time and location as the test organisms. The worm control sediment (Dillon Beach in Tomales Bay, CA) is sandy with some small gravel, and approximately 2.2% TOC. The control sediment for clams (Discovery Bay, WA) is of similar grain size, but lower in TOC, with a likely content of 1 – 1.5% TOC. The two sediments may be blended to create the control sediment that is suitable for both worms and clams.

3.1.3 Laboratory Bioaccumulation Test Procedures

The methods for bioaccumulation exposures will be similar to those of the EPA (USEPA 1993) and ASTM Method E1022-94, with the exceptions noted in this section.

The potential for the bioaccumulation of PCBs in tissues of clams and polychaete worms will be evaluated using 28-day sediment exposures with the clam *Mya arenaria* and the polychaete worm *Nephtys caecoides*. *Mya arenaria*[1] are a filter-feeding species found in the LDW and can be harvested by tribal members or recreational fishers. *Nephtys caecoides* are a free-burrowing, marine worm that engulfs sediment as it moves through bedded sediments and are representative of polychaete found in the marine portions of the LDW and Elliott Bay. The clams *Mya arenaria* will be supplied by Aquatic Research Organisms of Hampton, New Hampshire. *Nephtys caecoides* will be supplied by John Brezina of Dillon Beach, California.

Exposures of clams and polychaete worms will be conducted in the same test chamber. The polychaete *Nephtys caecoides* and clam species are often tested in the same chamber (DMMO

[1] *Mya arenaria* were the largest clam species and second most abundant in clam surveys conducted in the Lower Duwamish Waterway (Windward 2004).

User's Manual, USACE 2015); *Nephtys caecoides* is not a predator of clams. At test initiation (day 0), a total of twenty (20) adult *Nephtys* worms and five clams (adult, less than 2-inch length) will be placed into each core tube[2]. During test initiation, a subsample of five clams and 20 worms will also be randomly collected from the holding tanks for baseline tissue analysis. The baseline tissue subsample of worms and clams will be weighed in order to obtain an initial average tissue wet weight to compare against average tissue wet-weight values following sediment exposure. In conjunction with survival rates obtained from control sediment exposure, the wet-weight comparisons will help to understand the overall health of the test organisms. At the start of testing, the presence of native organisms in the sediment will be documented on the laboratory data forms. Water quality will be monitored daily throughout the 28-day test period for parameters including pH, salinity, temperature, and dissolved oxygen. Ammonia will be measured in overlying waters prior to test initiation, and again on Day 28 before test termination. If significant mortalities are observed, measured concentrations of ammonia will be assessed as to whether or not they may have contributed to the lethal effects. The target ranges for each of the water quality parameters are listed in Table 3.3. All water quality instruments will be calibrated daily or on their recommended schedule. Records of instrument calibration will be retained in the laboratory logs. Daily observations will include the number of live and dead organisms (as observed through visual observation), seawater flow rates, and any observed activity within the sediment cores, particularly related to the depth strata occupied by the test organism.

The test organisms are expected to remain in the top 6 inches (10 cm) due to their preference to remain in oxygenated sediment layers (this also occurs under field conditions). Additionally, the top 10-cm interval is the zone of compliance specified in the pilot study order, and is the layer being evaluated for porewater. The additional 6 to 12 inches of material in the core is provided to facilitate core collection. Although organisms are expected to stay at the test surface, in the event that organisms burrow into the native sediment, variability in the bioaccumulation results as a result of the extra material is unlikely. Once the organisms enter the native sediment, the mass transfer of PCBs to the organisms will not depend on the depth of the native sediment and instead will depend on the kinetics of PCB desorption from the native sediment and sorption into the

[2] The organism loading rates are based on the study design routinely used in the bioassay lab's standard bioaccumulation tests. The number of worms was based on the total volume of sediment in the test aquaria used in the standard tests; the number of clams was based on surface area of the test aquaria. The number of test organisms currently proposed provides more tissue mass than is needed for the chemical analysis, and allows extra tissue mass in case of any unexpected mortality.

The amount of natural carbon is expected to be sufficient for testing. Assuming that at least the top 1 inch of the 6-inch-diameter core reaches at least a 1% natural organic carbon content during the 3-year pilot study duration, at least 3 grams of organic carbon would be present, which is approximately 60 times the total mass of lipid present as a result of the 25 animals that will be added to the core. Based on the organic carbon contents observed in Monitoring Year 2 and 3, the number of animals can be revised to ensure we have sufficient organic carbon to support the animals in each chamber.

organisms. If significant burrowing into native sediment is observed, a discussion as to the potential impact to the study will be presented in the bioaccumulation testing report.

Any dead organisms on the surface will be removed from the test chambers. On Day 28, the sediment will be sieved to remove the clams and worms, recording the number of surviving organisms. Organisms observed other than the test organisms (particularly any predators) will be recorded. Surviving clams will be placed in clean flowing seawater in the absence of sediment to purge their gut contents over 24 hours. Surviving worms will be placed in control sediment (control sediment as described above) to purge their gut contents over 24 hours.

Following this 24-hour period, organisms will be processed to obtain tissue samples. Any sediment on the exterior surface of the test organisms will be rinsed; clams will be shucked. Tissue samples from each test chamber (up to 5 shucked clams or 20 individual worms per test chamber) will be placed into tared, certified-clean glass sample jars, weighed, frozen, and sent to the analytical laboratories via overnight courier. Tissues will be homogenized and then composited at the analytical laboratory to ensure that a similar mass of homogenized tissue from each test chamber is represented in the composite sample.

The following compositing scheme will be used to create six (6) clam tissue samples from the following sources:

- Clam tissue from the 6 “A” cores collected from the subtidal ENR plot;
- Clam tissue from the 6 “B” cores collected from the subtidal ENR plot;
- Clam tissue from the 6 “C” cores collected from the subtidal ENR plot;
- Clam tissue from the 6 “A” cores collected from the subtidal ENR+AC plot;
- Clam tissue from the 6 “B” cores collected from the subtidal ENR+AC plot; and
- Clam tissue from the 6 “C” cores collected from the subtidal ENR+AC plot.

The same approach will be used to create six (6) composite samples of worm tissue.

Occurrences of high mortality in any test replicate will be addressed on a case-by-case basis, in consultation with EPA and Ecology.

Forms: Daily water quality form, daily observation form, and Test termination form (Attachment A). Chain-of-custody forms will also be completed for transfer of the sample jars to the laboratories under custody (see Section 4.2 of the Pilot Study QAPP).

3.1.4 Laboratory Porewater Sampling

Dissolved PCB congeners in the sediment porewater during the 28-day exposures will be measured in top 10 cm of the cores using SPME fibers according to the same general approaches as used as the SPME deployments in the subplots as detailed in the Pilot Study QAPP (AMEC et al. 2016).

SPME fibers will be prepared in a manner consistent with the Pilot Study QAPP[3]. SPME fibers for deployment in the test chambers will be cut to 10-cm lengths (±0.5 cm). Eight fibers (80 cm total) will be placed in a 2- by 11-cm steel-mesh envelope (with 110-micrometer mesh openings) to protect the fibers from loss and breakage. The SPME envelopes (containing SPME fibers) will be rinsed in a 50:50 solution of acetonitrile and water, followed by three rinses with ultrapure water to remove trace impurities. The cleaned SPME envelopes will be placed in a solution containing performance reference compounds (PRCs), as detailed in the Pilot Study QAPP (AMEC et al. 2016). After a period of time sufficient to allow the PRCs to sorb to the polydimethylsiloxane (PDMS) coating on the SPME fibers (24 hours), the SPME envelopes will be blotted dry, wrapped separately in rinsed aluminum foil, and stored at 4°C until deployment.

On day 0 of the bioaccumulation test, prior to addition of organisms to the sediment cores, SPMEs will be deployed in the sediment core tubes. Immediately, but no more than 15 minutes before deployment, the SPME envelopes will be removed from cold storage, unwrapped from their aluminum foil layers, and attached to a corrosion-resistant steel plate or metal rod. One SPME envelope per sediment core will be inserted into the sediment such that the bottom of the envelope is approximately 10 cm (±1 cm) below the sediment-water interface. The SPMEs will remain embedded for the 4-week/28-day bioaccumulation test. In addition, on Day 0 of the bioaccumulation test, SPMEs will be deployed in a core containing laboratory seawater to allow a measurement of PCBs in a seawater-only test chamber concurrent with the 28-day tissue exposures, as further detailed in Section 3.3.1.2. On day 28 of the bioaccumulation test, during processing of the sediment for organism removal, SPME envelopes will removed from the sediment cores and overlying water core, removed from the steel plate or metal rod, wrapped individually in a layer of aluminum foil, placed in individual labeled sealable plastic bags, and

[3] SPME fibers are optical fibers composed of a 10-micrometer-thick polydimethylsiloxane (PDMS) coating around a 2000-micrometer-diameter silica core (Fiber-guide Industries, Stirling, New Jersey). The fibers will be cut to 10-centimeter lengths (±0.5 centimeters). For each core, one fiber will be placed in a 2-by-11-centimeter steel-mesh envelope (with 110-micrometer mesh openings) to protect the fiber from loss and breakage. The SPME envelopes (containing SPME fiber) will be rinsed in a 50:50 solution of acetonitrile and water, followed by three rinses with ultrapure water to remove trace impurities, and placed in a solution containing performance reference compounds (PRCs). The PRCs include one to two PCB congeners from each of the tri-, tetra-, penta-, hexa-, hepta-, and octa-chlorinated biphenyl homolog series.

stored at 4°C until processing and extraction. The SPME envelopes will be labeled with the sample ID (see Section 4.1 for naming protocols) and the date and time of collection.

The SPME fibers will be processed as soon as possible after the termination of laboratory bioaccumulation study but no later than 2 weeks after their retrieval from test chambers; processing will follow the methods described in Pilot Study QAPP. Under clean conditions in a laboratory, SPME fibers from the SPME envelopes will be composited to create seven (7) samples from the following sources:

- SPMEs from the 6 "A" cores collected from the subtidal ENR plot;
- SPMEs from the 6 "B" cores collected from the subtidal ENR plot;
- SPMEs from the 6 "C" cores collected from the subtidal ENR plot;
- SPMEs from the 6 "A" cores collected from the subtidal ENR+AC plot;
- SPMEs from the 6 "B" cores collected from the subtidal ENR+AC plot;
- SPMEs from the 6 "C" cores collected from the subtidal ENR+AC plot;
- SPMEs from the core with seawater only; and
- SPMEs from 18 SPME packets split equally into 3 trip blanks.

Following compositing of SPME fibers into the 10 pre-weighed vials (3 ENR composites, 3 ENR+AC composites, and 1 seawater only composite), vials will be weighed and extracted with solvent as described in Section 3.4.3 of the Pilot Study QAPP. Vials will be shipped to the analytical laboratory and stored at 4°C ± 2°C until further extract processing and analysis occurs at the analytical laboratory.

Forms: The analyst will complete a SPME preparation form (Pilot Study QAPP, Attachment A) for each batch of SPME fibers. The form will document the source of the base fibers, their purchase date, reference vendor-supplied information, reference to the analysis of the cleaned fiber, a list of the PRCs used and their concentrations in the soaking solutions, and a reference to the analysis of the PRC-loaded fiber. SPME deployment and recovery forms (Pilot Study QAPP, Attachment A) will be used to record the batch ID, discrete and composite sample IDs, and dates and times of deployment and retrieval for each sample. The compositing step will be documented on the SPME extraction and compositing form (Pilot Study QAPP, Attachment A).

3.1.5 Laboratory and Field Decontamination Procedures

Working surfaces, utensils, tools, equipment, mixing bowls, and other items that come in contact with the samples must have been cleaned before use, between composite samples, and between sampling events involving samples collected for chemical data. The decontamination procedure is as follows:

1. Prewash rinse with tap or site water.
2. Wash with solution of warm tap water or site water and detergent (e.g., Alconox).
3. Rinse with tap or site water.
4. Rinse thoroughly with laboratory-provided deionized water.
5. Store in a clean, closed container.

All dilute detergents, residual solvent (from the benthic sampling), and deionized rinsate will be captured separately in the field and in the laboratory and handled according to the procedures described in Section 3.2.8 of the Pilot Study QAPP.

3.2 ANALYTICAL METHODS

For the laboratory bioaccumulation study there will be two sample matrices that are being analyzed: tissues and the SPME extracts. Table 3.2 lists the methods, the sample preservation, the holding times, the minimum sample size, and the sample container preferred for shipment and storage. Tables 3.3 through 3.6 summarize the quality assurance goals (QAGs) for the samples collected for bioaccumulation tests and chemical analysis as described in this section.

3.2.1 Bioaccumulation Tests

The water quality parameters and target test conditions are summarized in Table 3.3.

In addition to the standard bioaccumulation test QA/QC measures, cores should be inspected for significant water loss during storage. Prior to placing on the water tables, each core should be inspected for any water loss or sediment disturbance. If either water loss or sediment disturbance is noted, the BTL should be notified to determine the suitability of the core for testing.

3.2.2 Tissues Analysis

Prior to analysis, the tissues in each individual replicate (test chamber) will be completely thawed and then homogenized (clam tissue samples will include siphon sheaths). Once homogenized, equal aliquots of each replicate will be combined in a sample composite following the compositing scheme described in Section 3.1.3.

The tissue composite samples will be analyzed for PCB congeners by high resolution gas chromatography with high resolution mass spectroscopy (HRGC/HRMS) following EPA Method 1668C. All 209 congeners will be reported in this project. Method 1668C defines QAGs for a subset of congeners rather than for all 209 congeners. Meeting the requirements for the subset of congeners is deemed by the method as sufficient to demonstrate acceptable performance for all

209 congeners. Per the method, internal standards and recovery standards will be used by the analytical laboratory for calibration to account for analyte loss during analysis.

Laboratory QA/QC requirements are presented in Table 3.6 and provided in Section 3.5. EPA Method 1668C contains extensive requirements for laboratory QC. These will be performed as required by the method and reported as part of the laboratory report (and in the electronic data deliverable [EDD]). A standard reference material (SRM), Lake Michigan Fish Tissue (Standard Reference Material 1947), will be run with each analytical batch to evaluate recovery of a similar matrix following Method 1668C. This SRM has been developed specifically to assess the accuracy and monitor the performance when analyzing chlorinated dioxins, furans, and polychlorinated biphenyl compounds. Advisory control limits have been established through round-robin testing for most dioxins/furans, CB congeners, and PCB Aroclor 1260. The SRM is stored at EPA's national Quality Assurance Technical Services contractor and permission to obtain the SRM will be obtained in coordination with the EPA Region 10 Manager. Lipids and percent solids will be analyzed by gravimetric measurement following methods in EPA 1668C. While there are no specific QA/QC requirements for lipids analysis, a laboratory duplicate sample will be included with the analytical batch to assess the variability of this measure.

3.2.3 SPME Porewater Sampler Extract Processing and PCB Congener Analysis

SPME fibers will be analyzed for PCB congeners only, following method 1668C. At the PCB congener laboratory, the SPME solvent extracts will be spiked with radio-isotope labeled-PCB analytical recovery standards and internal standards, and the extracts will be concentrated to a volume of approximately 100 microliters under a stream of nitrogen. This concentrated extract will be analyzed for PCB congeners, including the radio-isotope labeled congeners, using EPA Method 1668C. Because Method 1668 involves a significant amount of sample handling, reported concentrations are quantified using a combination of isotope dilution and internal standard correction. Details are contained in the method. Additional information on laboratory performance, QC, and reporting is provided in Section 3.5 of the Pilot Study QAPP.

3.2.4 Quality Assurance Criteria

The parameters used to assess data quality are precision, accuracy, representativeness, comparability, completeness, and sensitivity are described in detail in Section 3.4 of the Pilot Study QAPP.

3.3 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

This section presents an overview of the QA/QC information that will be used to track procedures in the field and lab. Table 3.6 summarizes the QA/QC samples by methods, but is not intended to capture to the full level of detail that is contained within the methods.

3.3.1 QC for Collection of Laboratory and SPME Samples

3.3.1.1 Tissues

The three exposure composites per subplot are equivalently representative of the subplot and, therefore, act as replicates and provide data regarding site heterogeneity and variability from sample handling. Additional sample volume will not be collected for matrix spike/matrix spike duplicates because EPA Method 1668 uses isotope dilution to measure the congener recovery from the matrix and as the sample moves through sampling handling steps. This is discussed more in Section 3.5 of the Pilot Study QAPP. As described below, two types of QC samples will be included in the laboratory bioaccumulation study: a native-sediment control and background tissues.

Native Sediment Control: Native sediment controls, sometimes referred to as a "negative control," will be tested concurrent to the test sediments. The primary purpose for the native sediment control is to ensure that test conditions are suitable for the survival of the test organisms during the 28-day exposure period. Sediment that is native to the test organisms will be provided by the test organism suppliers for use as control sediment. The control sediment will be placed into sediment cores similar to the cores used for the test sediments. While there is not a specific target survival level required for the bioaccumulation exposure, mean survival below 75% in the control (Inland Testing Manual, USEPA/USACE 1998) may provide an indication that the health of the test population is affected in some way.

Baseline Tissue: A subsample of tissues from each of the test species will be collected during test initiation for initial tissue concentrations. Baseline tissue samples will be analyzed for PCBs to determine the concentration of PCBs that may be present in the population of test organisms used in the bioaccumulation tests prior to test exposure. The baseline tissues will be depurated and preserved in a manner similar to the tissues exposed to the test treatments.

3.3.1.2 SPME Porewater Sampling

As with the tissue composites, the three composite samples per subplot act as replicates; therefore, no additional field duplicate will be necessary. Matrix spike and matrix spike duplicates are also not needed because of a combination of the PRCs used to access recovery from the fiber and the use labeled congeners in EPA Method 1668 to monitor congener recoveries. As described below, two types of QC samples will be collected for SPMEs: seawater blank and trip blanks.

SPME Seawater Blank: A seawater blank is needed for the SPME fibers can accumulate very low levels of PCBs that may be present in the laboratory seawater from the Hood Canal. SPMEs will be exposed to laboratory seawater in a seawater-only test chamber concurrent to the 28-day tissue exposures. This chamber will contain at least 6 times the volume of water as present in the

overlying water of the sediment cores, and will contain 6 SPME envelopes that will be composited into a single composite SPME sample. This chamber will also be aerated and receive the same seawater replacement configuration as the sediment cores. After the 28-day exposure period, SPME fibers from the seawater blank will be processed and analyzed in a manner identical to the SPME fibers exposed to test sediments.

SPME Trip Blanks: Trip blanks are needed for the SPME fibers because their high sorption capacity makes field contamination prior to and after deployment a concern. For the laboratory test initiation, three trip blank composites will be created from 18 SPME envelopes. The trip blanks are created at the same time and using the same methods as the SPME samplers. These trip blanks will be unwrapped from their foil, and exposed to laboratory air for approximately 5 minutes. After exposure, the envelope will be wrapped in rinsed aluminum foil and stored at 4°C ± 2°C. Within 2 weeks, the trip blank fibers will be processed and extracted.

3.3.2 Sample Delivery Group

Traditionally, a sample delivery group (SDG) is defined as no more than 20 samples or a group of samples received at the laboratory within a 2-week period. For this project, the following SDGs are defined:

Event	Basis for SDG	Expected Sample Count
Laboratory Bioaccumulation Study	1 SDG for clam tissue samples	6 + 1 baseline
	1 SDG for polychaete worm tissue samples	6 + 1 baseline
	1 SDG for composite SPME extracts	6 + 1 Seawater blank + 3 Trip Blanks

3.3.3 Laboratory QA/QC Criteria

The analyst will review the results of QC analyses (described below) from each SDG immediately after a SDG has been analyzed. The QC sample results will then be evaluated to determine whether control limits have been exceeded. If control limits have been exceeded in the sample group, the project QAO will be contacted immediately, and corrective action, such as method modifications followed by reprocessing of the affected samples, will be initiated before a subsequent group of samples is processed.

The QC procedures and sample analyses to be performed by the analytical laboratory will be consistent with those described in Section 3.5 of the Pilot Study QAPP and are summarized in Table 3.6. The associated control limits for precision and accuracy for PCBs in tissue are summarized in Table 3.4 through 3.6. SPME extract chemistry limits are detailed Table 3.5 of the Pilot Study QAPP.

In addition to the QC samples discussed in Table 3.6, there are QA/QC measures conducted in support of the bioaccumulation exposures. These include water quality measurements and negative control treatments.

The integrity of the sediment strata and the porewater should be maintained during the transport and handling of the sediment cores from the field and throughout the bioaccumulation exposures. In the field, the chief scientist should inspect the core to verify that a "sediment plug" of native sediment is present under the ENR or ENR+AC layer. The material should be fine-grained in nature and should be sufficient to prevent the loss of porewater from the overlying ENR/AC layer. Cores with signs of disturbance, voids, or lack of an intact layer at the bottom of the core should be rejected.

Once the cores are sealed, there should be negligible water loss from the bottom of the core. Signs of water loss may be pooling water at the bottom of the core or a decrease in the water level at the top of the core. Cores showing a significant loss of water should be inspected to ensure the caps are not leaking.

Water quality monitoring of test chambers provides documentation that testing parameter limits for each procedure were met throughout the duration of the test. Routine calibration of measurement probes per manufacturer's instructions ensures consistency in readings. Measurements are taken daily or as proscribed by the test procedure. An individual test may be acceptable even if temperature, dissolved oxygen, pH, or salinity fall outside recommended specifications, depending on the degree of deviation. When feasible, corrective actions are performed to bring an out-of-range observation back to within parameters without adversely influencing the test (e.g., temperature control or aeration addition). These actions are taken immediately upon discovery and are considered standard procedure. Recommended ranges are typically well within the natural tolerance limits of the test organisms. When deviations occur, test acceptability falls to the experience and professional judgment of the technical director and the permitting authority responsible for accepting the test results.

The control sample is a "negative control" for the matrix tested. Sediment shown to support the health and survival of the test organisms is used as the control sediment. The negative control is included for each test to evaluate test performance and the health of the specific batch of test organisms. The target for survival in the bioaccumulation tests is 75% based on guidance from the USEPA/USACE (1998). Failure of a control sample to meet the test criteria may indicate that the test organisms were insufficiently healthy to provide a valid test or that procedural problems existed that would invalidate the test results. Because the primary goal of the bioaccumulation exposures is sufficient tissue for chemical analysis, control survival below 75% does not indicate that the exposures are invalid. In the event that mean control survival falls below the target, a

potential cause of excess negative response should be identified. This will include a review of the water quality observations, daily test observations, and any other useful anecdotal observations.

3.4 FIELD INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Field instrument and equipment testing, inspection and maintenance in support of the laboratory bioaccumulation study will follow the methods described in Section 3.6 of the Pilot Study QAPP.

3.5 LABORATORY INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

Laboratory instrument calibration for the analytical and biological testing laboratories will be conducted in accordance with the QC requirements identified in the manufacturers' instructions and the laboratory standard operating procedures (SOPs). General requirements are detailed in Section 3.7 of Pilot Study QAPP.

3.6 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

The Field Coordinator will be responsible for ensuring that all supplies necessary to conduct the sampling, including collecting, processing, and transporting samples, are available and in good working order at the beginning of the field work. The Field Coordinator will monitor supplies and equipment throughout the sampling and replenish or replace them as necessary.

Likewise, the laboratory managers are responsible for ensuring that all supplies necessary to perform the analyses are available and uncontaminated, that equipment is in good working order and conforms to the QA protocols, and that the procedures, including the laboratory's QA plan are documented and followed.

3.7 DATA REDUCTION

Procedures regarding data reduction will follow the methods described in Section 3.9 of the Pilot Study QAPP.

3.8 DATA AND RECORDS MANAGEMENT

Procedures regarding data records and management will follow the methods described in Section 3.10 of the Pilot Study QAPP, with the following addition.

3.8.1 EcoAnalysts Laboratory for Bioaccumulation Test Records

Archived information and access logs are protected against fire, theft, loss, environmental deterioration, vermin, and in the case of electronic records, electronic or magnetic sources. All electronic records are backed-up daily (onsite) and weekly (offsite storage). Access to protected records is limited to laboratory management or their designees to prevent unauthorized access or amendment. Records are disposed according to applicable regulation, client request, or after

five years. For this project, the contract with the laboratory will require that records be retained for 10 years.

4.0 SAMPLE HANDLING AND CUSTODY DOCUMENTATION

This section discusses sample handling and chain-of-custody documentation, including sample nomenclature; sample chain of custody; sample preservation, handling, and transport; and sample receipt procedures.

4.1 SAMPLE NOMENCLATURE

Sample nomenclature is defined for the SPME porewater samples, the surface sediment cores, and the tissue samples.

Each sample will be assigned a unique alphanumeric ID number that will consist of seven to nine components identifying various aspects of the sample, with each component separated by a hyphen ("-"). The hyphen will allow for ease in electronic data entry from the field forms into the database.

The sample ID components are summarized in Table 4.1.

Table 4.1 Tissue and SPME Porewater Sample ID Components

Order	Component	Definition
1st	Project area	"LDW" = Lower Duwamish Waterway
2nd	Monitoring event	"LBS" = Laboratory Bioaccumulation Study
3rd	Plot type	"SU" = subtidal plot "CON" = control sediment "WAT" = the seawater control
4th	Subplot	"ENR" = enhanced natural recovery only "ENR+AC" = enhanced natural recovery with activated carbon
5th	Grid cell number	A single number between 0 and 6: "0" = composite "1" to "6" = indicates a un-composited sample collected from the grid cell indicated by the number
6th	Composite number	This is a two-character code for the composite number: "CA," "CB," or "CC"

Table 4.1 Tissue and SPME Porewater Sample ID Components

Order	Component	Definition
7th	Sample medium	"CORE" = sediment core tube (used for chain of custody between field collection and laboratory, and in the laboratory test) "S010" = SPME fibers in envelopes and vials collected from 0 to 10 cm (used for chain of custody between sampler and preparation laboratory) "CLAM" = clam tissue "WORM" = worm tissue
8th (as needed)	Collocated sample	Single-digit numbers from 1 to 5, if needed to ensure that enough volume is available for analysis, collocated cores or SPME envelopes may be collected
9th (as needed)	QC sample	"TB" = trip blank (SPME) "BAS" – baseline tissue

Abbreviations:
SPME Solid-phase microextraction

The first component of the sample ID will represent the LDW project area ("LDW"). The second component will represent the monitoring event ("LBS"). The third through sixth components will represent the location (plot and subplot) and the type of sample (tissue, SPME). The seventh component will represent the sample medium. The laboratory will use "CLAM", "WORM", and "S010" on sample chain-of-custody forms. The last two ID components will be needed only under certain conditions. The eighth component will allow the collection of collocated sediment cores or SPME fibers to increase the sample volume, and the ninth component will represent the QC samples.

4.2 SAMPLE CHAIN-OF-CUSTODY

The laboratory bioaccumulation study will follow the sample chain-of-custody procedures described in Section 4.2 of the Pilot Study QAPP.

4.3 SAMPLE PRESERVATION, HANDLING, AND TRANSPORT

This section discusses sample preservation, handling, and transport of tissue samples for PCB and lipids analysis and SPME samples for PCB analyses.

4.3.1 Tissue Samples

At the testing laboratory, tissue samples from each individual test chamber will be placed in pre-cleaned, laboratory-provided 16-oz wide-mouth amber glass jars leaving a minimum of approximately 1 cm of headspace to prevent breakage during shipping and storage. The sample containers will be stored in a freezer at approximately -20°C until frozen.

Samples jars must be appropriately labelled with waterproof, self-adhering labels. Each sample label will contain the project number, sample identification (Section 4.1), preservation technique, analyses, date, and time of collection, and initials of the person(s) filling the sample jar. A completed sample label will be affixed to each sample container. The labels will be covered with clear tape immediately after their completion to protect them from stains or deterioration due to water and sediment.

Samples will be shipped via overnight delivery in accordance with state and federal regulations as well as U.S. Department of Transportation (DOT) standards as outlined in the Pilot Study QAPP.

4.3.2 SPME Extracts for PCB Analysis

The transport of SPME fibers from the field to the extraction laboratory is discussed in Section 3. The two vials with fibers and solvent will be wrapped in bubble wrap and shipped in a cooler containing double-bagged wet ice at 4°C with sufficient cushioning material. The samples will be shipped in accordance with state and federal regulations as well as DOT standards using the same procedures as those detailed in Section 4.3 of the Pilot Study QAPP.

4.4 SAMPLE RECEIPT

Refer to the Pilot Study QAPP for a description of the sample receipt requirements for this program.

5.0 ASSESSMENT AND OVERSIGHT

Refer to the Pilot Study QAPP for a description of the assessment and oversight requirements for this program.

6.0 REPORTING AND RECORD RETENTION

6.1 DATA SUBMITTALS AND MONITORING REPORTS

Reporting associated with the laboratory bioaccumulation study will evaluate the bioaccumulation potential of PCB in tissues and concurrent SPME fibers exposed to ENR+AC compared to ENR alone in the subtidal subplots.

Validated sampling and test data will be provided to EPA and Ecology as an appendix to the Year 3 Monitoring Report. The validated sampling data will be provided in two formats: (1) printed compilation in the Year 3 Report and (2) LDWG database format. The LDWG database format will contain the sample coordinates cross referenced against the sample location and sample IDs.

The laboratory bioaccumulation study results, including the test performance, tissue and porewater concentrations observed in the test treatments will be included in the Year 3 monitoring report. The monitoring reports will be submitted to EPA and Ecology initially in draft form for their review.

6.2 RECORD MAINTENANCE AND STORAGE

Refer to the Pilot Study QAPP for a description of records maintenance and storage measures for this program.

7.0 REFERENCES

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TABLES

Table 3.1
Location-Specific Information for Year 3

Subtidal Plot: East Lane				Subtidal Plot: West Lane			
Treatment: ENR				Treatment: ENR+AC			
Year 3	Comp A	Comp B	Comp C	Year 3	Comp A	Comp B	Comp C
Cell 1	2	14	22	Cell 1	3	24	22
Cell 2	17	21	4	Cell 2	5	16	21
Cell 3	17	24	2	Cell 3	3	13	15
Cell 4	24	15	6	Cell 4	2	4	1
Cell 5	13	6	17	Cell 5	22	5	1
Cell 6	4	19	17	Cell 6	3	5	11

Abbreviations:

Comp	Composite
ENR	Enhanced
ENR+AC	Enhanced natural recovery amended with activated carbon

Table 3.2
Analytical Parameters, Methods, Laboratories, Sample Containers, and Sample Preservation

Parameter	Analytical Method	Laboratory	Sample Preservation	Technical Holding Time	Minimum Sample Size	Sample Container(s)
Sediment Cores for Bioaccumulation Testing						
Bioaccumulation Tests	USEPA 1993; ASTM E1022-94	Ramboll Environ	2 - 6°C	8 weeks	12" core	Core tube
Tissue Samples						
PCB congeners	EPA 1668C	Frontier	Transport: less than 6°C. Storage: less than -10°C.	1 year	6 grams	16-oz. AWMG jar
Percent Lipids						
Total solids						
SPME Fiber Extracts						
PCB congeners	EPA 1668C	Frontier	Transport: less than 6°C. Storage: less than 4°C.	1 year	Entire hexane extract	2-mL amber glass vial

Abbreviations:

Frontier	Frontier Analytical Laboratory	mL	Milliliter
ASTM	American Society for Testing and Materials	oz	Ounce
AWMG	Amber, wide-mouth glass with teflon-lined lid	PCB	Polychlorinated biphenyl
°C	Degrees Celsius	SPME	Solid-phase microextraction

Table 3.3
Methods and Acceptable Quality Assurance Goals for Biological Testing

Parameter	Analytical Method	Lab	Control Survival	Water Quality					Overlying Water Exchange Rate	Photoperiod[1]
				Temperature °C	Salinity ppt	DO mg/L	pH	Total Ammonia mg/L		
Bioaccumulation Test	USEPA 1993; ASTM E1022-94	EcoAnalysts Port Gamble	≥75%	15 ±2	32 ±2	>4.5	Ambient ±0.5	Undefined[2]	5-8 L/d or 600-1000% of volume/d	16:08

Notes:

1. Photoperiod is reported as hours of light:hours of dark
2. Total ammonia values in the overlying water will be recorded for each core tube at test initiation and termination

Abbreviations:

ASTM	American Society for Testing and Materials
°C	Degrees Celsius
DO	Dissolved oxygen
mg/L	Milligrams per liter
ppt	Parts per thousand

Table 3.4
Methods and Acceptable Quality Assurance Criteria for Chemistry in Tissue Samples

Parameter	Analytical Method	Lab	Units	Sensitivity [1]		Precision[2]	Accuracy[3]	Completeness[4]
				RL/LOQ	MDL/LOD			
PCB Congeners	EPA 1668C	Frontier	pg/g dw	4	See Table 3.5	50%RPD	40-140%R	95%
Lipids	EPA 1668C	Frontier	%	NA	NA	NA	NA	95%

Notes:

1. Sensitivity is assessed by the use of initial and continuing calibration and laboratory control samples.
2. Precision is assessed by the use of laboratory control samples and laboratory duplicates.
3. Accuracy is assessed by calibration, laboratory control samples, and matrix spikes/matrix spike duplicates.
4. Completeness is measured as the number of results that are acceptable for use vs. the number of samples analyzed.

Units:

pg/g dw	Picograms per gram dry weight
%	Percent

Abbreviations:

Frontier	Frontier Analytical Laboratory
EPA	U.S. Environmental Protection Agency
LOD	Limit of detection
LOQ	Limit of quantification
MDL	Method detection limit
NA	Not applicable
PCB	Polychlorinated biphenyl
R	Recovery
RL	Reporting limit
RPD	Relative percent difference

Table 3.5
Methods and Acceptable Quality Assurance Goal for PCB Congeners in Tissue

PCB Congener by Frontier Using EPA 1668C		Tissue Samples			Acceptance Criteria for Native PCBs					Acceptance Criteria for Labeled PCBs					
			Sensitivity: Analysis of 5-Gram Sample[2]				IPR					IPR			
	Co-elution[2]	Units	RL/LOQ	MDL/LOD	Test Conc.	VER Recovery	RSD	Mean Recovery	OPR Recovery	Test Conc.	VER Recovery	RSD	Mean Recovery	OPR Recovery	Sample Recovery
PCB-1	NA	pg/g dw	4	0.06	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-3	NA	pg/g dw	4	0.06	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-4	NA	pg/g dw	4	0.39	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-15	NA	pg/g dw	4	0.22	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-19	NA	pg/g dw	4	0.15	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-28	NA	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	65 -135%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-37	NA	pg/g dw	4	0.09	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-54	NA	pg/g dw	4	0.10	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	70%	20 - 135%	15 - 145%	5 - 145%
PCB-77	NA	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-81	NA	pg/g dw	4	0.13	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-104	NA	pg/g dw	4	0.12	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-105	NA	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-106	118	pg/g dw	4	0.12	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-111	115	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	75 -125%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-114	NA	pg/g dw	4	0.13	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-118	106	pg/g dw	4	0.12	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-123	NA	pg/g dw	4	0.13	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-126	NA	pg/g dw	4	0.09	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-155	NA	pg/g dw	4	0.1	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-156	NA	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-157	NA	pg/g dw	4	0.13	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-167	NA	pg/g dw	4	0.12	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-169	NA	pg/g dw	4	0.09	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-178	NA	pg/g dw	4	0.19	50	75 - 125%	25%	70 - 130%	60 - 135%	100	75 -125%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-188	NA	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-189	NA	pg/g dw	4	0.08	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-202	NA	pg/g dw	4	0.11	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-205	NA	pg/g dw	4	0.06	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-206	NA	pg/g dw	4	0.09	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-208	NA	pg/g dw	4	0.07	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%
PCB-209	NA	pg/g dw	4	0.05	50	75 - 125%	25%	70 - 130%	60 - 135%	100	50 -145%	50%	45 - 135%	40 - 145%	10 - 145%

Notes:

1. Method 1668 defines quality assurance goals for the subset of congeners above rather than for all 209 congeners. All 209 congeners will be reported in this project. Meeting the requirements for the congeners tabulated above is deemed by the method as sufficient to demonstrate acceptable performance for all 209 congeners.
2. Chromatographic co-elution occurs when two (or more) compounds do not chromatographically separate due to the fact that both species have retention times that differ by less than the resolution of the method. The concentration is reported for the first co-eluting congener only. All co-eluting congeners receive a qualifier that indicates the congener that receives the value.

Abbreviations:

Conc. Concentration
EPA U.S. Environmental Protection Agency
IPR Initial precision and recovery
LOD Limit of detection
LOQ Limit of quantification
MDL Method detection limit
NA Not applicable
OPR Ongoing precision and recovery
PCB Polychlorinated biphenyl
pg/g dw Picograms per gram dry weight
RL Reporting limit
RSD Relative standard deviation
SPME Solid-phase microextraction
VER Calibration verification

Table 3.6
Laboratory QA/QC Requirements

Parameter	Analytical Method	Initial Calibration	Continuing Calibration	Method Blanks	Laboratory Control Samples	Sample Matrix Quality Control			Surrogate Spikes
						Matrix Spike	Matrix Spike Duplicate	Laboratory Duplicate or Triplicate	
Sediment									
PCB congeners [2]	EPA 1668C	Prior to analysis	Every 10 to 20 analyses or 12 hours	1 per batch[1]	1 per batch[1]	Handled by use of isotope dilution in EPA 1668C		NA	Each sample
Lipids		NA	NA	NA	NA	NA	NA	1 per batch[1]	NA
Percent Solids		NA	NA	NA	NA	NA	NA	NA	NA
SPME Fiber Extracts									
PCB congeners	EPA 1668C	Prior to analysis	Every 10 to 20 analyses or 12 hours	1 per batch[1]	1 per batch[1]	Handled by use of isotope dilution in EPA 1668C		NA	Each sample

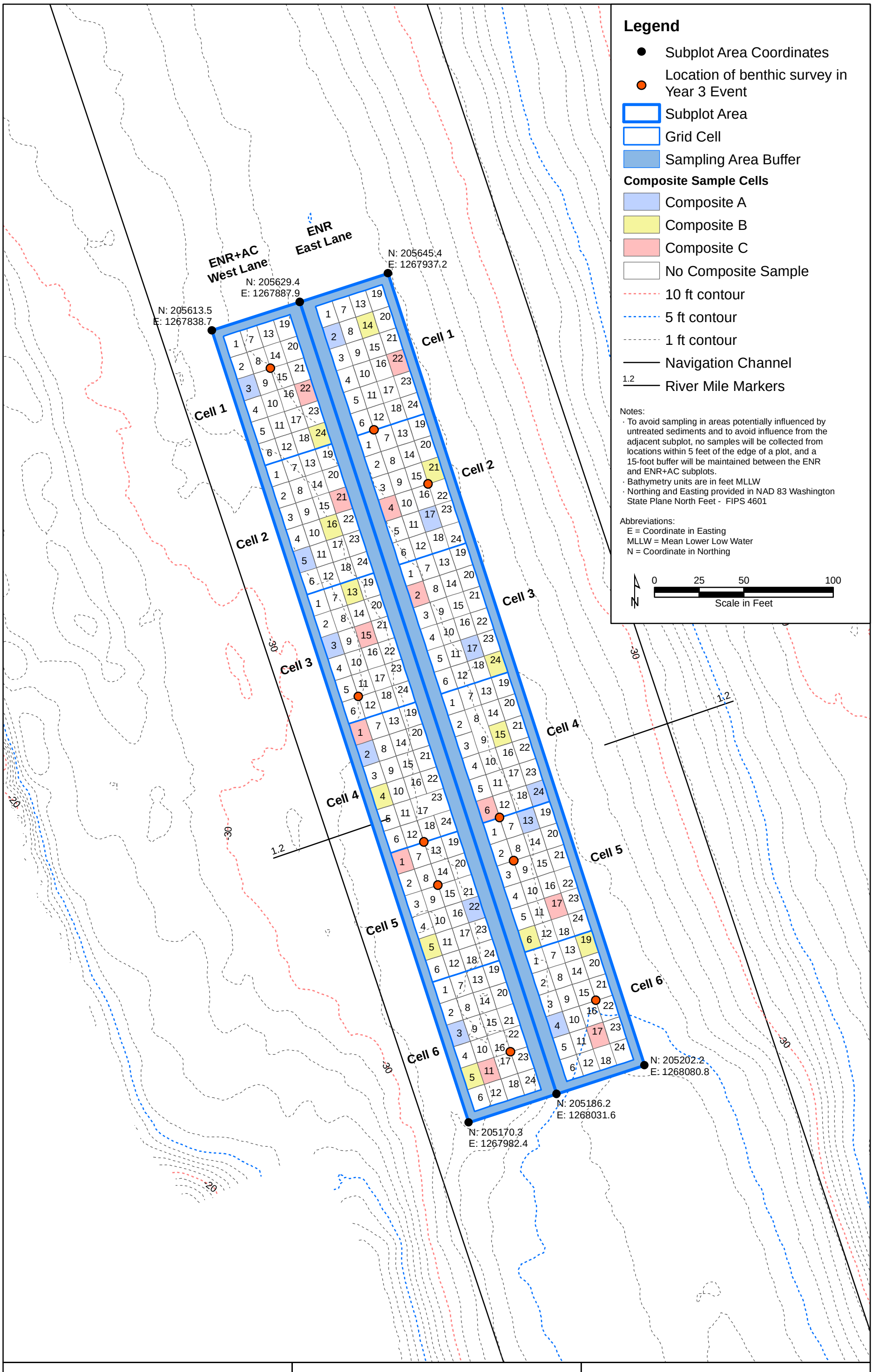
Notes:

1. Project SDGs are expected to range in size from 1 sample to 20 samples. Batches are groups of 20 or fewer samples that move through sample preparation and analysis together. A batch formed at the lab may include samples from more than one SDG, and the SDGs in a batch may be from multiple projects. In the table above "per SDG" indicates that the "batch" QC must be run on a sample from the SDG from the project.
2. Includes analysis of Lake Michigan Fish Tissue Standard Reference Material (Standard Reference Material 1947) run with each analytical batch.

Abbreviations:

EPA U.S. Environmental Protection Agency
PCB Polychlorinated biphenyl
QA/QC Quality assurance/quality control
SDG Sample Delivery Group
SPME Solid-phase microextraction
NA Not applicable

FIGURE



Lower Duwamish Waterway Group
Port of Seattle / City of Seattle / King County / The Boeing Company

ENR/AC Pilot Study
Lab Bioaccumulation QAPP Addendum
Lower Duwamish Waterway

Figure 3.1
Subtidal Plot
Sample Composite Grid

I:\GIS\Projects\AMEC-KC-ENR\MXD\Document 2015_0422\Figure 3.1 Subtidal Plot with Sample Composite Grid - Year 3.mxd
5/3/2016

ATTACHMENT A

Laboratory Forms

28 DAY BIOACCUMULATION WQ DATA SHEET

CLIENT	PROJECT	SPECIES	LABORATORY	PROTOCOL
LDWG	Lower Duwamish Pilot Study	*Mya arenaria and Nephtys caecoides*	Port Gamble	EPA 1993
PROJECT NUMBER	**PROJECT MANAGER**	**WATER DESCRIPTION**	**TEST START DATE**	**TEST END DATE**
0	0	North Hood Canal; filtered		

WATER QUALITY DATA

TEST CONDITIONS				DO (mg/L) >4.0		TEMP (C) 15±2		SALIN.(ppt) 32±2		pH Ambient ± 0.5				
Sample ID	DAY	REP	JAR #	D.O.		TEMP		SALINITY		pH		TECHNICIAN	FLOW	Total Ammonia (mg/L)
				meter	mg/L	meter	°C	meter	ppt	meter	unit			
	0	1												
		2												
		3												
		4												
		5												
	1	1												
	2	2												
	3	3												
	4	4												
	5	5												
	6	1												
	7	2												
	8	3												
	9	4												
	10	5												
	11	1												
	12	2												
	13	3												
	14	4												

28 DAY BIOACCUMULATION WQ DATA SHEET

CLIENT	PROJECT	SPECIES	LABORATORY	PROTOCOL
LDWG	Lower Duwamish Pilot Study	*Mya arenaria and Nephtys caecoides*	Port Gamble	EPA 1993
PROJECT NUMBER	**PROJECT MANAGER**	**WATER DESCRIPTION**	**TEST START DATE**	**TEST END DATE**
0	0	North Hood Canal; filtered		

WATER QUALITY DATA

TEST CONDITIONS				DO (mg/L) >4.0		TEMP (C) 15±2		SALIN.(ppt) 32±2		pH Ambient ± 0.5				
Sample ID	DAY	REP	JAR #	D.O.		TEMP		SALINITY		pH		TECHNICIAN	FLOW	Total Ammonia (mg/L)
				meter	mg/L	meter	°C	meter	ppt	meter	unit			
	15	5												
	16	1												
	17	2												
	18	3												
	19	4												
	20	5												
	21	1												
	22	2												
	23	3												
	24	4												
	25	5												
	26	1												
	27	2												
	28	1												
		2												
		3												
		4												
		5												

28-DAY BIOACCUMULATION TEST OBSERVATION DATA SHEET (DAYS 0-14)

Client	Project	Project No.	Project Manager	Laboratory	Protocol	Species
LDWG	Lower Duwamish Pilot Study			Port Gamble	EPA 1993	*Mya arenaria and Nephtys caecoides*

Observation Key

#S= Number on the Surface
#M= Number of Mortality
L=Anoxic Surface
F=Fungal Patches
D=No Air Flow (DO?)
N=Normal
B=No Burrows
#C/W=Number clams /worms below ENR

INITIAL # OF ORGANISMS

ENDPOINT DATA & OBSERVATIONS

Sample ID	REP	Position	Initial # if Different	Day	DATE / TECHN.												
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
			1															
			2															
			3															
			4															
			5															
			6															
			7															
			8															
			9															
			10															
			11															
			12															
			13															
			14															
			15															

28-DAY BIOACCUMULATION TEST OBSERVATION DATA SHEET (DAYS 0-14)

Client	Project	Project No.	Project Manager	Laboratory	Protocol	Species
LDWG	Lower Duwamish Pilot Study			Port Gamble	EPA 1993	*Mya arenaria and Nephtys caecoides*

Observation Key

#S= Number on the Surface
#M= Number of Mortality
L=Anoxic Surface
F=Fungal Patches
D=No Air Flow (DO?)
N=Normal
B=No Burrows
#C/W=Number clams /worms below ENR

INITIAL # OF ORGANISMS

ENDPOINT DATA & OBSERVATIONS

Sample ID	REP	Position	Initial # if Different	Day	DATE / TECHN.												
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
			16															
			17															
			18															
			19															
			20															
			21															
			22															
			23															
			24															
			25															
			26															
			27															
			28															
			29															
			30															

28-DAY BIOACCUMULATION TEST OBSERVATION DATA SHEET (DAYS 15-28)

Client	Project	Project No.	Project Manager	Laboratory	Protocol	Species	
LDWG	Lower Duwamish Pilot Study			Port Gamble	EPA 1993	*Mya arenaria and Nephtys caecoides*	

Observation Key

#S= Number on the Surface
#M= Number of Mortality
L=Anoxic Surface
F=Fungal Patches
D=No Air Flow (DO?)
N=Normal
B=No Burrows
#C/W=Number clams /worms below ENR

INITIAL # OF ORGANISMS

ENDPOINT DATA & OBSERVATIONS

Sample ID	REP	Position	Initial # if Diffe	DATE / TECHN. Day	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Number Remaining
		1																	
		2																	
		3																	
		4																	
		5																	
		6																	
		7																	
		8																	
		9																	
		10																	
		11																	
		12																	
		13																	
		14																	
		15																	

Note position # and enumerate any non-test species:

28-DAY BIOACCUMULATION TEST OBSERVATION DATA SHEET (DAYS 15-28)

Client	Project	Project No.	Project Manager	Laboratory	Protocol	Species	
LDWG	Lower Duwamish Pilot Study			Port Gamble	EPA 1993	*Mya arenaria and Nephtys caecoides*	

Observation Key

#S= Number on the Surface
#M= Number of Mortality
L=Anoxic Surface
F=Fungal Patches
D=No Air Flow (DO?)
N=Normal
B=No Burrows
#C/W=Number clams /worms below ENR

INITIAL # OF ORGANISMS

ENDPOINT DATA & OBSERVATIONS

Sample ID	REP	Position	Initial # if Diffe	DATE / TECHN. Day	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Number Remaining
		16																	
		17																	
		18																	
		19																	
		20																	
		21																	
		22																	
		23																	
		24																	
		25																	
		26																	
		27																	
		28																	
		29																	
		30																	

Note position # and enumerate any non-test species: