FINAL

Attachment C
Updated Standard Operating Procedures

The standard operating procedures (SOPs) included in this attachment are updates of those provided in Appendix E of the Pre-Design Investigation quality assurance project plan (Windward and Anchor QEA 2022). Therefore, original numbering and lettering from that appendix have been retained to allow for direct comparison.

SOP Number E2
10-Day Acute Sediment Toxicity Test with Marine Amphipods (PSEP)

1.0 SCOPE

This test determines the short term, adverse effects of potentially contaminated sediment on marine amphipods. Sediment toxicity testing will be conducted according to procedures outlined in **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995)**, including modifications from the Sediment Management Annual Review Meeting (SMARM) clarification papers. Other references include **USACE/USEPA (1991) (OTM)** and **USACE/USEPA (1998) (ITM)** (for dredged sediments), **ASTM E 1367**, **EPA 600/R-01/020**, and **EPA/600/R-94/025**.

2.0 SUMMARY OF TEST

2.1 Approach

Table 1. Conditions for Performing 10-Day Solid Phase Toxicity Testing on Marine Amphipods

Test type	Static Non-renewal*
Test duration	10 Day
Lighting	Ambient and Constant
Test chamber size	1-L glass beaker
Test sediment depth	2 cm (~175 mL)
Test solution volume	775 mL (Chamber Vol. up to 950 mL)
Renewal of test solution	None*
No. of organisms per chamber	20
No. of replicates per treatment	5 test replicates 2 sacrificial chambers (one being the water quality surrogate) recommended minimum
Feeding	None
Test solution aeration	Trickle-flow (sufficient to maintain DO levels above 60% saturation, mg/L limits in Table 2)

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) or ammonia.

2.2 Physical Requirements

Table 2. Species Specific Test Condition Summaries

Species	*Ampelisca Abdita* (preferred, when percent fines >60%)[1]	*Rhepoxynius abronius*	*Eohaustorius estuarius* (preferred, when percent fines <60%)[1]	*Leptocheirus plumulosus*[5]
Life Stage Tested	Immature amphipods	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 2-4 mm, mixed sexes
Feeding	Will not be fed	Will not be fed	Will not be fed	Will not be fed
Temperature (°C)	20 ± 1	15 ± 1	15 ± 1	25 ± 2
Salinity (ppt)	28 ± 1	28 ± 1	28 ± 1 or ambient[4]	28 ± 1
pH	7-9	7-9	7-9	7-9
DO (≥ 60% Saturation)	4.6 mg/L	5.1 mg/L	5.1 mg/L	4.4 mg/L
Grain Size [1]	> 60% fines	< 60% fines	< 60 % fines preferred (may tolerate 0 to 90% fines); Provided clay fraction < 20%[2]	5 to 100% fines, < 85% clay[7]
Un-ionized Ammonia[3] (mg/L)	< 0.236[6]	< 0.4	< 0.8	< 0.8
Hydrogen Sulfide[3] (mg/L)	0.0094[6]	0.099	0.122	0.122

[1] Grain size distributions are recommended guidelines in the PSEP guidance and should not be considered absolute criteria. Species selection generally includes discussion with regulatory agencies and share holders and can be chosen exclusive from grain size characteristics (i.e. comparison to historical data with same species, species availability, etc.). If *Ampelisca* are not available, *Eohaustorius* may be used provided that percent fines are <90% and clay content is known to be <20%[2]. Alternatively, *Leptocheirus* may be substituted for *Ampelisca* given agency approval.

[2] Kendall and McMillan 1999

[3] Inyoue et al. 2015

[4] Test salinity for *E. estuarius* may be conducted at the interstitial salinity (ambient) of the test sediments. The target test salinity should be approved by the client or regulatory agency, and will vary agency depending upon the objectives of the testing program.

[5] Direct guidance for *L. plumulosus* is not given under PSEP guidelines; however, test conditions are similar to that of *E. estuarius* and described in other guidance documents (EPA 1991, 1994).

[6]Ampelisca unionized ammonia and hydrogen sulfide limits are for overlying water, all other amphipod limits are for porewater

[7]USEPA 2001

3.0 TEST ORGANISM

The test organism should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions, ecological importance, and ease of handling in the laboratory. Ideally, organisms with wide geographical distribution should be selected so test results can be compared among laboratories with similar organisms. Test conditions for each amphipod species are summarized in Tables 1 and 2.

Table 3. Test Organism Suppliers

Species	*Ampelisca abdita*	*Rhepoxynius abronius*	*Eohaustorius estuaries*	*Leptocheirus plumulosus*
Life Stage Tested	Immature amphipods, or mature females only	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 3-5 mm, mixed sexes
Sources	John Brezina and Associates, Dillon Beach, CA; Aquatic Research Organisms, Hampton, NH	John Brezina and Associates, Dillon Beach, CA; or field collected	Northwest Amphipod, Newport, OR	Aquatic BioSystems, Fort Collins, CO; Aquatic Research Organisms, Hampton, NH

3.1 Test Organism Care

Records will be kept, including the date and location collected, feeding regime, and sediment characteristics.

Holding time for amphipods is standardized to between 2 and 10 days.

4.0 TEST SUBSTANCE

The test sediments will be labeled, properly stored, and tracked by internal chain-of-custody procedures throughout its tenure at the facility. The sediments will not be heated, filtered, distilled, frozen, or otherwise altered without prior written consent by the Client. The test substance is stored at 0 – 6 °C in the dark, in a secure and distinct storage area. Containers should also have as little air as possible over the sediment or be stored with nitrogen gas in the overlying head space.

Test sediments should not be sieved prior to testing unless there is potential concern of similar species, competitors, or predators. Native sediments should always be sieved to remove amphipods from the material to be used as the Control treatment. A 0.5 mm sieve is sufficient to remove the amphipods and sediments should only be dry sieved (manually pushed through the

sieve) using only the water present in the sample. These procedures can be performed prior to test set-up and stored under the conditions described above.

5.0 EQUIPMENT

5.1 Instrumentation/Equipment

Microprocessor-controlled recorder, and a digital thermometer
Light meter
DO meter and probe
Salinity meter and probe
pH meter and probe
Ammonia probe meter and ancillary supplies
Sulfide probe & meter or spectrophotometer, and ancillary supplies
Microbalance capable of measuring weights to the nearest 0.0001 mg
Environmental test chamber or water bath capable of maintaining test temperature within 1ºC
1 L and 250 mL test chambers
Clean 0.45-µm filtered seawater
Deionized water
Pipettes
Miscellaneous labware (wash bottles, tally counters, culture bowls, etc.)
500 µm stainless steel sieves
Holding cups (food grade plastic is acceptable)
Stir plate and teflon stir bars
Centrifuge and tubes for collecting pore water

5.2 Apparatus

5.2.1 Test Area

The test area consists of a water bath or temperature controlled room with constant monitoring of test temperature and appropriate illumination. The facility will be well ventilated and free of fumes.

5.2.2 Lighting

Overhead lighting will be ambient and continuous (24-hour).

5.2.3 Test Chambers

1-L glass jars with a 10-cm internal diameter, covered with a petri dish.

6.0 PROCEDURE

6.1 Preparation

6.1.1 Labware Preparation

Labware is described as any plastic or glass material used in the laboratory that will come into contact with any of the test substances or organisms in this evaluation. Labware must be cleaned prior to use following the procedures outlined in SOP ***LAB019***.

6.1.2 Dilution Water Preparation

Natural seawater will be obtained from North Hood Canal, sand filtered, and filtered to 0.45µm. Seawater will be adjusted as necessary to maintain a target test salinity. Salinity should be lowered with the addition of high purity deionized water or increased with the addition of bioassay grade sea salts or brine.

6.1.3 Test Organism Acclimation

For acclimation, amphipods will be held in control sediment with salinity adjusted dilution water. Gentle aeration will be provided for the duration of the acclimation period. Two to three days are sufficient for acclimation to the test conditions. Organisms may be fed a slurry of ground alfalfa or Tetramin™ if held for an extended period.

Amphipods in holding containers will be checked daily before the initiation of a test. Individuals that emerge from the sediment and appear dead or unhealthy will be discarded. If greater than 10% of the amphipods die or appear unhealthy during 48 hours preceding the test, the health of the batch of organisms should be evaluated for use in the proposed testing. This may include an additional day of holding to determine if mortalities or abnormal behavior are due to shipping or acclimation stress, and not indicative of an overly sensitive population.

6.2 Primary Task

6.2.1 Pre-Test Analyses

Prior to test initiation, and preferably as soon as sediments are received at the testing facility, porewater should be collected from a homogenized sample from each sediment treatment (including reference and controls). This sample should be analyzed for interstitial salinity, pH, total ammonia, and sulfides. Unionized ammonia is to be calculated from the measured total ammonia and pH and the test target temperature and salinity. The parameters listed in Table 3

are recommendations based upon the tolerance of each species. If conditions within the sediment are outside the tolerance ranges, the project manager and/or client should be notified and possible corrective actions discussed. The most common corrective action involves test chamber overlying water renewal or purging to bring test conditions with tolerance ranges. These procedures are described further in Section 6.2.3. Note that the limits for *Ampelisca abdita* are for overlying water, as their burrow construction exposes them to overlying water more than porewater. If ammonia, sulfides, and/or salinity in the porewater are outside of the limits in Table 4, it may be necessary to set up a mock test jar to test the overlying water before proceeding to purging or acclimation renewals. This is also recommended for the other species as well, as the addition of test water could decrease the porewater values upon equalization.

Table 4. Species Specific Ammonia and Sulfide Limits[1]

Species	*Ampelisca abdita*	*Rhepoxynius abronius*	*Eohaustorius estuarius*	*Leptocheirus plumulosus*[2]
Matrix	Overlying Water	Porewater	Porewater	Porewater
Un-ionized Ammonia (mg/L))	< 0.236	< 0.4	< 0.8	< 0.8
Hydrogen Sulfide (mg/L)	0.0094	0.099	0.122	0.122

[1] Inouye et al. 2015.
[2] Direct guidance for *L. plumulosus* is not given under PSEP guidelines; however, test conditions are similar to that of *E. estuarius* and described in other guidance documents (EPA 1991, 1994).

6.2.2 Test Sediment Addition

Test sediment will be prepared using glassware cleaned according to Section 6.1.1, pre-cleaned glassware of a disposable nature, or non-toxic food grade plastic. All test chambers should be labeled accordingly with corresponding random number positions. After setup, the test chambers are distributed throughout the testing area based upon their position numbers. All 5 treatment replicates, including the corresponding Water Quality Surrogate (see Section 6.2.3), should be included in the randomized test matrix.

If necessary, sieving of the control sediment and/or test treatments will be performed (see Section 4.0). On the day before the test begins, each test sediment sample will be thoroughly homogenized within its storage container, and an aliquot added to a test chamber depth of 2 cm.

The sediment within the test chamber will be settled by tapping the test chamber against the side of the hand. Prepared seawater is gently added up to the 950-mL level (about 775 mL). A solid disk attached to a rod is placed inside the chamber to limit the suspension of the sediment into the water column by diffusing the water down the inside of the test chamber. The disc should be maintained just above the water surface as the test chamber is filled. The sample is left

overnight with gentle aeration to allow suspended particles to settle and equilibrium to be established between sediment and overlying water before the amphipods are added.

6.2.3 Sample Adjustments

If the water quality conditions in the test chamber are not suitable to support the selected amphipod species, it may be necessary to adjust those conditions to within tolerance limits. The two most common parameters which may require attention include interstitial salinity and ammonia. Water quality conditions (exclusive of contaminants) should be within the tolerance limits of the test species to remove the impact of their interference on the determination of toxic effects. Depending upon the program, manipulations to the test treatments may be performed to correct any deviations. Unfortunately, these manipulations may also alter the level of contaminants through purging or alter their available chemical state (salinity or pH change). Best professional judgment must be employed when deciding to manipulate the sample treatments and should always involve discussion with the client or regulatory agency. If manipulations are performed to the test treatments, the associated Control and Reference sediment should be treated in the same manner.

Generally, adjustments to the interstitial salinity of the sediments are not desirable. Exceptions to this may be sediments with very low interstitial salinities that are destined for ocean disposal. If sediments are from freshwater, follow the acclimation procedures in SOP *LAB064*. If salinity is only slightly out of range, the salinity may be adjusted by replacing the overlying water within the test chambers with water of salinity equal to, or slightly greater than (or slightly less than if lowering), the target test salinity. The test chamber water should be removed through siphoning or pumping the water out to a level just above the test sediment. Care should be taken not to remove any sediment during this process. Prepared seawater is gently added up to the 950-mL level. A solid disk attached to a rod is place inside the chamber to limit the suspension of the sediment into the water column by diffusing the water down the inside of the test chamber. The disc should be maintained just above the water surface as the test chamber is filled.

For sediments with ammonia or sulfide concentrations exceeding those values listed in Table 4, purging may be required to bring the test chambers conditions within acceptable limits. In most cases this should be determined in the pre-test pore water analyses (Section 6.2.1). General procedures for purging of the test chambers are described in further detail in the SMARM clarification paper “Ammonia and Amphipod Toxicity Testing” (Barton 2002). Additional sacrificial surrogate chambers should be created to monitor pore water ammonia levels during the acclimation process. Overlying water exchanges are conducted in the same manner as the overlying water renewal for salinity adjustment described above. Purging should be conducted twice daily until the pore water ammonia or sulfide concentrations are below the threshold values. Control and reference sediment must undergo purging alongside test sediments (Inouye et al 2015). Porewater ammonia and/or sulfide levels, along with pH, should be monitored every

1-3 days during the purging process. Overlying ammonia and/or sulfide levels, along with pH, should also be measured as part of the monitoring procedure as it gives an estimate of ammonia reduction without the breakdown of a surrogate chamber. Once the porewater ammonia has been reduced below the threshold values, purging should be terminated, and the testing period can commence. Depending upon the program, purging may or may not be continued after test initiation. It may be possible in highly biogenic sediment that ammonia may increase again over the course of the test if renewals are discontinued.

6.2.4 Reference Toxicity Test

During this 96-hour toxicity test with marine amphipods and a test substance, five concentrations of a reference substance (ammonium chloride) with 10 test organisms will be used to assess the health of the test organisms. Three test chambers per reference concentration may be used. One concentration will be the 96-hour LC_{50}. The other four concentrations will be selected to bracket the LC_{50}. The LC_{50} values will be compared with historical data from definitive bioassays with the reference substance. The results of the 96-hour mortality, determined during this study, will be reported and used in combination with control mortality to characterize the health of the test organisms. Table 5 summarizes the test conditions for conducting a 96-hour water-only reference toxicant test.

Table 5. Conditions for Performing 4-Day Water-Only Reference Testing on Marine Amphipods

Test type	Static Non-renewal
Test duration	4 Day
Lighting	Dark and Constant[1]
Test chamber size	250-mL glass beaker (minimum)
Test solution volume	200-mL (minimum)
Renewal of test solution	None
No. of organisms per chamber	10 recommended (minimum of 5)
No. of replicates per treatment	3
Feeding	None
Test solution aeration	None unless needed to maintain DO levels above 60% saturation

[1] In the absence of sediment, amphipods will continue to attempt to bury into the bottom of the chamber. Keeping the amphipods in the dark will lessen this digging behavior thus reducing undue stress on the test organisms.

The results of the ammonia reference-toxicant may be compared to the ammonia concentrations observed within the test samples to assist in correlating any ammonia related effects within a specific batch of organisms. Table 4 summarizes the published threshold ammonia concentrations for each species.

6.2.3 Reference and Control Sediment

During this 10-day toxicity test with marine amphipods on project sediment(s), reference sediment(s) will be used to provide a site-specific basis for comparison of potentially toxic and non-toxic conditions. Control sediment, collected during amphipod collection at the same site, will be used to determine the condition of the amphipods.

6.2.5 Water Quality

During routine test observations, a daily record of test room or water bath temperatures and test chamber aeration should be made.

In order to limit the impact of disturbance on the test organisms, all water quality measurements during the testing procedure will be performed in a surrogate water quality only chamber. In addition to the five test treatment replicates, a minimum of two additional surrogate chambers should also be tested; one for use as a water quality surrogate (WQS), and one to be utilized at test initiation for porewater analyses. The WQS will be used at termination for porewater analyses. Surrogate chambers should be treated in the same manner as the test replicates. This includes randomization among the test treatments and addition of test animals. Additional pre-test surrogate chambers may also be required to monitor pore water salinity, ammonia, or sulfide manipulations.

After one day of acclimation after sediment and overlying water layering (test day 0), an initial set of water quality parameters will be measured in the overlying water of the WQS for each test treatment. The water quality parameters include temperature, dissolved oxygen (DO), pH, salinity, total ammonia, and total sulfides, following guidance outlined in SOPs *EQP060*, *EQP061*, *EQP062*, *LAB063* and either *LAB017* or *LAB018*. In addition, a surrogate replicate from each test treatment will be sacrificed in order to extract porewater via centrifugation for subsequent analysis of ammonia and sulfides. Prior to test initiation, these initial water quality measurements must be reviewed to ensure that they are within the testing parameters. Test initiation should be postponed until any deviations are addressed and corrected.

On test days 1 through 9, temperature, DO, pH, and salinity will be measured in the water quality surrogate chamber of each treatment. At test termination (test day 10) the full suite of measurements will be repeated as on day 0.

6.2.6 Test Organism Addition

Amphipods are sieved from the holding sediment (500 µm sieve) and transferred to a sorting tray containing water of the holding temperature and salinity. Active, healthy amphipods are randomly selected from the sorting tray and sequentially distributed among dishes containing

approximately 15 mL of dilution seawater until each cup contains 5 individuals. Prior to addition to the test chambers, the number of organisms is verified by recounting the individuals within the cup as well as confirming health and appearance. Unacceptable amphipods are discarded and replaced prior to introduction.

Twenty animals (4 cups of 5 animals each) are then added to the randomly positioned test chambers. Addition should occur with minimal disruption of the sediment by gently pouring the water and amphipods from the sorting cups into the test chamber. Any amphipods remaining in the cup should be gently washed into the test chamber. After addition, the test chamber is marked to confirm organism addition, re-covered, and aeration restored. Any amphipods that do not bury within 15 minutes will be removed and replaced (*Ampelisca abdita* should be allowed one hour for burial).

6.2.5 Test Initiation

The test initiation time is when the test organisms are distributed to the first test chamber.

6.2.6 Test Observations

Notes are made on sediment appearance and unusual conditions. This can include fungal and algal growth. The number of amphipods that have emerged from the sediment, either floating on the water surface or lying on top of the sediment is recorded. Amphipods that are floating on the surface can be released from the surface tension by dropping a small drop of water (from the test chamber) with a pipette. Care must be taken not to cross-contaminate beakers. Dead animals either on the water or sediment surface are not removed during the exposure period. A list of observation types and their corresponding codes are detailed in Table 6.

Table 6. Observation Key for Recording Test Observations

Normal	N
Number of bodies on surface (mortality). Can indicate a corpse or a molt.	#M
Number emerged (actively swimming in water column, or walking on sediment surface; not burrowing)	#E
Number floating on surface. Animals caught in surface tension of water.	#FOS
Growth. Indicative of fungal, algal, or bacterial mats	G
No Air Flow	D
Water too cloudy/turbid for observation	TC
Anoxic surface	L

6.3 Post-task

The bioassay is terminated on day 10. After final observations are performed, the contents of each test chamber are sieved through a 0.5-mm sieve. A gentle spray of seawater is used to wash the sediment through the sieve. Material retained on the sieve is transferred to a clean sorting vessel containing seawater of a similar salinity and temperature as the test. The numbers of live and dead amphipods are recorded. An amphipod is considered alive if there is any sign of movement (e.g., pleopod twitching or response to gentle prodding). Recoveries may not equal 20 due to the decomposition of dead animals through the test. Although not commonly conducted, there is also a procedure for evaluating the ability of the amphipods (excluding *A. abdita*) to rebury into Control sediment. This sublethal endpoint is discussed in further detail in PSEP 1995.

Results Needed:

- Percent mortality for each treatment
- Mean water quality values by treatment
- LC_{50} and 95% confidence limits (for ref. tox.)
- Reburial

In screening tests, the responses of amphipods in collected test sediments are compared to control and reference site sediments.

6.4 Reporting

The report may include, but will not be limited to, the following:

- Name and address of the laboratory conducting the study, and dates on which the study was initiated and completed.
- The name of the project manager, other scientists or professionals, and supervisory personnel involved in the study.
- Objectives as stated in the protocol.
- A description of the methods used.
- Transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusion drawn from the analysis.
- The test substance identified by code number and the date each sample was used.
- The number of organisms used in the study.
- Concentrations of exposure and exposure method.

- Any circumstances that may have affected the quality or integrity of the data, including deviations from test protocols or Standard Operating Procedures.
- The location where raw data and the final report will be stored.
- Additions or corrections to a final report will be in the form of an amendment by the Project Manager. The amendment will clearly identify that part of the final report that is being altered and the reason(s) for the alteration(s). The amendment will be signed and dated by the Project Manager.

The master copy of the final report will be signed and dated by the Project Manager.

7.0 HEALTH AND SAFETY CONSIDERATIONS

Proper laboratory protection, including lab hood or ventilation system, lab coat, closed-toe shoes, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

Refer to the Port Gamble Laboratory's Chemical Hygiene Plan and Health and Safety Plan at S:\Health and Safety for procedures to ensure safe operation in the laboratory and for contingency plans in the event of an accident or emergency.

For specific chemical health and safety information, refer to the Safety Data Sheet log.

8.0 PERSONNEL

Any laboratory personnel demonstrating competence with this method may perform the procedure.

9.0 QUALITY ASSURANCE REQUIREMENTS (ACCEPTANCE CRITERIA)

This study will be conducted according to the Standard Operating Procedures of the Port Gamble Laboratory which are in effect during the time the study is being performed. In the case where there is a conflict between the other SOPs and this protocol, the protocol will be the definitive procedure.

Usually tests would be unacceptable if the following conditions occurred:

- More than 10% of the organisms die in the Control treatment.

Test data will need to be evaluated and qualified if:

- All test chambers were not identical.

- Treatments were not randomly assigned to test chambers.
- Test organisms were not randomly or impartially distributed to test chambers.
- All test animals were not from the same population, were not all of the same species, or were not of acceptable quality.
- Reference sediment and controls were not included in the test.
- Amphipods were maintained in the laboratory for less than two days or greater than ten days, unless the effect of prolonged maintenance in the laboratory has been shown to have no significant effect on sensitivity.
- Temperature, DO, pH, salinity, sulfides and ammonia were not measured, or were not within acceptable range.
- Test organisms were not acclimated at the test temperature and salinity at least 24 hours before they were placed in test chambers.
- Aeration to the test chamber was off for an extended time such that the DO levels dropped below acceptable limits and was associated with mortality.
- Response criteria were not monitored in a random fashion.

10.0 REFERENCE DOCUMENTS

10.1 Internal Documents

SOP EQP060 : Calibration/Operation/Maintenance of Orion Star Model A329 pH/ISE/Conductivity/ DO Meter (pH)
SOP EQP061: Calibration/Operation/Maintenance of Orion Star Model A329 pH/ISE/Conductivity/DO Meter (DO)
SOP EQP062: Calibration/Operation/Maintenance of Orion Star Model A329 pH/ISE/Conductivity/DO Meter (Conductivity and Temperature)
SOP LAB019: Labware Washing Procedures
SOP LAB063: Sampling and Measurement of Total Ammonia in Water
SOP LAB017: Sulfide Sampling With Ion Selective Probe
SOP LAB018: Sulfide Sampling and Measurement with Spectrophotometer
SOP LAB064: Acclimation of Sediments for Bioassay Testing

10.2 External Documents

Inouye, Laura; Erika Hoffman; David Fox. 2015. DMMP Clarification Paper: Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing for Marine Bioassays. August 14, 2015.

Kendall, David; Russ MacMillan. 1999. DMMP Clarification Paper: Clarification on the Use of the Amphipod, *Eohaustorius estuarius*, Relative to Grain Size and Salinity. October 20, 1999.

ASTM E 1367-03. "Standard Test Method for Measuring the Toxicity of Sediment Associated Contaminants with Estuarine and Marine Invertebrates." Annual Book of Standards. Volume 11.06 "Biological Effects and Environmental Fate; Biotechnology." American Society of Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA. 2014.

Puget Sound Water Quality Authority. Revised July 1995. "Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments." Prepared for U.S. EPA Region 10, Office of Puget Sound. Seattle, W A.

USACE/USEPA (U.S. Army Corps of Engineers/U.S. Environmental Protection Agency). 1991. Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual." Office of Water, Washington, DC. EPA/503/8-91/001. February, 1991.

USACE/USEPA. 1994. "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods." Office of Research and Development, Washington, DC. EPA/600/R-94/025.

USACE/USEPA. 1998. "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual." Office of Water, Washington, DC. EPA/823/B-98/004. February, 1998.

USEPA. 2001. "Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus*". First Edition. Office of Research and Development, Western Ecology Division, Newport, OR.; Office of Water, Washington, D.C.; Engineer Research and Development Center, Waterways Experiment Staton, U.S. Army Corps of Engineers, Vicksburg, MS. EPA 600/R-01/020. March, 2001.

11.0 APPENDIX OF CHANGE

- 08/20/15 Changed test organism acclimation section to reflect a 10% mortality threshold in assessing the organism's health

- 05/23/16 Added "uncontrolled" statement to SOP and updated Health and Safety section

- 05/09/17 Updated health and safety information, removed branding, added review documentation section. Added "proprietary information" statement to footer.

- 04/28/21 Updated formatting throughout. Added % clay preferences, updated sulfide and ammonia limits in Table 2. Updated organism suppliers in Table 3. Updated instrument list. Added references to internal documents. Section 6.2.1/Table 4 updated with new ammonia and sulfide limits, added setup of mock jars for more accurate estimation of porewater levels in test. Clarified purging method in section 6.2.3. Removed Reference toxicant ammonia limit table (lab always run ammonia toxicants, so not needed). Clarified time of initiation in section 6.2.5. Updated observation key in Table 6.

- 4/25/23 Updated grain size requirements in Table 2. Updated salinity requirement for *Leptocheirus* in Table 2. Added reference to acclimation SOP in section 6.2.3. Updated reference list.

12.0 DOCUMENT REVIEW

SOP Approval

Name	Signature	Title	Date
		Quality Systems Director	
		Laboratory Manager	

Acknowledgement below indicates that the individuals have read and understood the concepts summarized in this document.

Name	Signature	Date

SOP Number E4
20-Day Chronic Growth and Survival Test with *Neanthes arenaceodentata*

1.0 SCOPE

To determine the chronic toxicity of marine sediments on the marine polychaete *Neanthes arenaceodentata*. Sediment toxicity testing will be conducted according to guidelines presented in **ASTM E1611**, **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995)**, and the various updates presented during the Annual Sediment Management Review meetings (SMARM Clarification Papers).

2.0 SUMMARY OF TEST

Table 1 Test Condition Summary

Sample storage conditions	4°C, dark minimal head space
Recommended Sediment Holding Time:	≤8 weeks (56 days)
Test Species	***Neanthes arenaceodentata***
Age class	Juvenile (2-3 weeks post-emergence)
Test Procedures	ASTM, PSEP 1995 with SMARM revisions
Regulatory program	SMS, DMMP, SEF or other as mandated by the associated program
Test type/duration	20-Day static renewal
Test chamber	1-Liter glass beaker or jar
Exposure volume	175 mL (2cm) sediment/ 775 mL water
Replicates per treatment	5 + 2 surrogate chambers (one used for WQ measurements throughout the test)
Control / Diluent water	North Hood Canal, 0.45 µm filtered
Test Lighting	Continuous
Aeration	Continuous from test initiation: 100 bubbles per minute
Test temperature	Recommended: 20 ± 1 °C
Test salinity	Recommended: 28 ± 2 ppt
Test dissolved oxygen	Recommended: > 4.6 mg/L (60% saturation @ 20°C and 28 ppt salinity)[1]
Test pH	Recommended: 7 – 9 [2]
Unionized ammonia	< 0.46 mg/L porewater
Hydrogen sulfide	< 3.4 mg/L in porewater
Organisms/replicate	5
Feeding	40 mg/jar every other day (8 mg/ind every other day)
Water renewal	Water renewed every third day (1/3 volume of exposure chamber)

[1] PSEP guidance is not specific on dissolved oxygen limits. The value of 60% saturation is based on ASTM 2006.
[2] pH is monitored as a water quality parameter. There are generally no control limits for pH; however measurements of pH may be useful in interpreting results (Ecology 2003).

2.2 Physical Requirements

DO	>4.6 mg/L (60% Saturation)
Temperature	20 ± 1°C
Salinity	28 ± 2 ppt (PSEP); 28 – 36 ppt (ASTM)
pH	7.0 - 9.0
Lighting	Continuous ambient light at approximately 50-100 foot-candles (550-1050 lux)

2.3 Biological Requirements

Feeding	Organisms will be fed ground TetraMin® on an every-other-day basis. The amount of food provided will be approximately 8 mg (dry weight) per juvenile *N. arenaceodentata*
Life stage	Juvenile worms (2-3 weeks, 0.25 - 1.0 mg dry weight)

3.0 TEST ORGANISM

The test organism should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions, ecological importance, and ease of handling in the laboratory. Ideally, organisms with wide geographical distribution should be selected so test results can be compared among laboratories with similar organisms. The organism for this protocol is *Neanthes arenaceodentata*.

3.1 Test Organism Specifications

Species:	*Neanthes arenaceodentata*
Source:	Aquatic Toxicology Support, Bremerton, WA, or other suitable supplier
Age:	Juvenile Worms (2-3 weeks, 0.25-1.0 mg dry weight), laboratory cultured

4.0 TEST SUBSTANCE

The test substance will be labeled, properly stored, and tracked by internal chain-of-custody procedures throughout its tenure at the Port Gamble Laboratory. The test substance will not be heated, filtered, distilled, frozen, or otherwise altered without prior written consent by the Client. The test substance is stored at 0 - 6°C in a secure and distinct storage area.

5.0 EQUIPMENT

5.1 Instrumentation/Equipment

Thermometer
Light meter
DO meter and probe
Salinity meter and probe
pH meter and probe
Ammonia probe meter and ancillary supplies
Microbalance capable of measuring weights to the nearest 0.01 mg
Environmental test chamber or water bath capable of maintaining 20 ± 1°C
1000 mL test chambers
Clean filtered seawater
Deionized water
Pipettes
Brushes
Miscellaneous labware (wash bottles, tally counters, culture bowls, etc.)
500 µm stainless steel sieves
Aluminum weigh boats
Holding cups (food grade plastic is acceptable)
Stir plate and teflon stir bars
Finely ground TetraMin®
Centrifuge and centrifuge Teflon® tubes for collecting pore water
Drying oven capable of maintaining 60°C
Muffle furnace capable of 550°C
Desiccator

5.2 Apparatus

5.2.1 Test Area

The test area consists of a water bath or a room with constant temperature and appropriate illumination. The facility will be well ventilated and free of fumes.

5.2.2 Lighting

Continuous overhead lighting will be at 50-100 foot-candles (550-1050 Lux).

5.2.3 Test Chambers

1000 mL glass beakers with a 10 cm internal diameter covered with a petri dish.

6.0 PROCEDURE

6.1 Preparation

6.1.1 Labware Preparation

Labware is described as any plastic or glass material used in the laboratory that will come into contact with any of the test substances or organisms in this evaluation. Labware must be cleaned prior to use following the procedures outlined in SOP ***LAB019***.

6.1.2 Dilution Water Preparation

Natural seawater will be obtained from North Hood Canal, sand filtered, and filtered to 0.45µm. Seawater will be adjusted as necessary to maintain a target test salinity of 28 ppt. Salinity should be lowered with the addition of high purity deionized water or increased with the addition of bioassay grade sea salts or brine.

6.1.3 Test Organism Care and Acclimation

Upon receipt, salinity and temperature of water in shipping containers should be measured. If salinity is more than 2 ppt different from the target test salinity of 28 ppt then the salinity should be adjusted (no more than 3 ppt daily). If salinity is outside the range of 15 to 35 ppt, then test animals may be possibly stressed and the supplier should be notified to provide a new batch of test organisms. Temperature should be allowed to equilibrate to test temperature prior to removing animals from shipping containers. If temperature of shipping containers is outside the range of 15 to 25°C then a new batch of test organisms may be required. Animals should be held for at least 24 hours prior to testing and may be fed during holding period.

If animal health is suspect upon receipt (e.g. over 10% of number received dead, animals behaving strangely or diseased), notify the laboratory manager who will assess whether to notify the supplier and order replacements. If more than 10% of the organisms die in the 48h prior to testing, the entire batch is discarded, and a new batch is ordered. If the acclimation process is repeated with a new group of test organisms and excessive mortality occurs, an alternative source of dilution water should be used.

6.2 Primary Task

6.2.1 Test Sediment Addition

Test sediment will be prepared using labware cleaned according to Section 6.1.1, pre-cleaned labware of a disposable nature, or non-toxic food grade plastic.

If pre-sieving of sediment is required to exclude large material or potential predators they may be press sieved (no water) through a clean stainless steel sieve (2 mm mesh).

One day prior to test initiation test sediment, reference, and control sediment should be added to the test chambers. Sediment should be thoroughly homogenized prior to addition to the test chambers. Approximately 2 cm of sediment should be added to each of the 5 replicate containers and applicable surrogate chambers. Once sediment has been added, clean filtered seawater should be added up to the 950-mL mark at a salinity of 28 ppt. Water should be added to ensure minimal disturbance of test sediments. Test chambers should be aerated at approximately 100 bubbles/minute under test temperature and photoperiod regime. The system should be left overnight with gentle aeration to allow suspended particles to settle and an equilibrium to be established between sediment and overlying water before the organisms are added.

6.2.2 Reference and Control Sediment Test

During this 20-day toxicity test with *Neanthes arenaceodentata* and a test sediment, a reference substance will be used to provide a site-specific basis for comparison of potentially toxic and non-toxic conditions.

6.2.3 Reference Toxicity Test

During this 96-hour toxicity test with *Neanthes arenaceodentata* and a test substance, five concentrations of a reference substance (ammonium chloride) with 10 test organisms will be used to assess the health of the test organisms. Three test chambers per reference concentration may be used. One concentration will be the 96-hour LC_{50}. The other four concentrations will be selected to bracket the LC_{50}. The LC_{50} values will be compared with historical data from definitive bioassays with the reference substance. The results of the 96-hour mortality, determined during this study, will be reported and used in combination with control mortality to characterize the health of the test organisms. Table 2 summarizes the test conditions for conducting a 96-hour water-only reference toxicant test.

Table 2. Conditions for Performing 4-Day Water-Only Reference Testing on *Neanthes arenaceodentata*

Test type	Static Non-renewal
Test duration	4 Day
Toxicant	Ammonium chloride
Lighting	Continuous, 50 to 100 f.c.
Test chamber size	250-mL glass beaker (minimum)
Test solution volume	200-mL (minimum)
Renewal of test solution	None
No. of organisms per chamber	10 recommended (minimum of 5)
No. of replicates per treatment	3
Feeding	None
Test solution aeration	None unless needed to maintain DO levels above 4.6 mg/L

The results of the ammonia reference-toxicant may be compared to the ammonia concentrations observed within the test samples to assist in correlating any ammonia related effects within a specific batch of organisms.

6.2.4 Test Organism Addition

For test initiation, worms should be selected at random from a large culture dish(es) that contains all of the shipped animals. Animals should be added in order of random number, not treatment, to ensure an equal distribution of selected animals across treatments (i.e., so that animals selected initially aren't all in a single treatment and animals selected at the end aren't all in a single treatment). Transfer of animals to the test chambers is accomplished by gently drawing one worm into the wide end of a Pasteur pipette and adding the organism directly to the test chamber just above the water's surface to prevent cross-contamination. The number of animals added will be tracked by a counter operated by the person adding the worms. As animals are added to the test chamber, test chambers should be marked. Test chambers should be observed within one hour of addition. Worms demonstrating non-burrowing behavior may be replaced, if the observer believes the behavior results from factors other than sediment toxicity.

During test initiation, five worms should be assigned to an additional 3 holding cups for initial calculated individual weight measurements. Worms for these measurements should be selected at random from the culture dish and should be collected at regular intervals during initiation of the test so as not bias the initial size measurements.

6.2.5 Test Initiation

The test is initiated when test organisms are distributed completely to each test chamber. The test initiation time is when organisms are introduced to the first chamber.

To make initial weight measurements, individual animals should be gently scooped onto a small brush, rinsed briefly in deionized water, blotted dry on a Kimwipe and transferred onto a pre dried, pre weighed, pre marked (number etched into pan prior to pre weighing) aluminum pan (2x2 cm piece of aluminum foil). All five worms from one holding cup should be placed onto a foil weigh boat. Fold pans over to prevent loss of animals over the course of drying. Oven dry worms and pans at 60°C for 24 hours prior to weighing. Remove pans/worms from the oven and place in a desiccator for approximately 1 hour to cool to room temperature. All weight measurements must be made on a balance that can be measured to the nearest 0.01 mg.

An initial ash-free dry weight (AFDW) measurement on the worms is necessary if this endpoint is included in the final test weight determinations. After obtaining the dry weight data, each of the weigh boats is then dried in a muffle furnace heated to 550°C for 2 hours in order to determine ashed weights. The ashed boats are again weighed to 0.01 mg and the ashed weight is subtracted from the dry weight to calculate the AFDW.

6.2.6 Test Maintenance

Feed worms every 48 hours. TetraMin® should be provided at approximately 8 mg (dry weight) per juvenile *Neanthes* (40 mg per test chamber).

Overlying water should be renewed every three days (total of six renewals). Approximately one third of the overlying water volume should be exchanged at each renewal.

6.2.7 Test Measurements

Data are recorded on data sheets.

Water Quality. A daily record of test room or water bath temperatures and test chamber aeration should be made. Water quality measurement should be made prior to renewals. Record temperature, salinity, dissolved oxygen, and pH in one randomly selected test chamber per treatment or a designated water quality surrogate chamber. Overlying and porewater ammonia and sulfides are measured in a surrogate chamber at test initiation and termination. Follow procedures outlines in SOPs ***EQP060, EQP061, EQP062, LAB063*** and either ***LAB017*** or ***LAB018***

Note: If the unionized ammonia is ≥ 0.46 mg/L or the hydrogen sulfide is ≥ 3.4 in the porewater, purging procedures are required before the test is initiated.

Biological. Response criteria indicating toxicity of test sediment include mortality, sublethal and chronic effects. A sublethal effect is the emergence from highly toxic sediment during the course of the test. Chronic effects are monitored by comparing the differences in dry weight or AFDW between test sediments and reference sediments (or control treatment when appropriate).

Mortality. At test termination (Day 20), all sediment from each individual chamber should be sieved through a 500 µm sieve to collect surviving organisms. Gently rinse sediment through sieve using 26 - 30 ppt salinity seawater. Gently remove animals from the sieve using a camel hair brush taking care not to damage the animal. Once removed, the animal should be placed into a labeled holding container containing clean filtered seawater (26 - 30 ppt) at room temperature. Record whether animal recovered from each test chamber is surviving, dead, or missing (for purposed of calculations all missing animals are assumed to be dead).

Growth (Dry Weight). Growth is measured by the dry weight of the surviving test worms within a replicate. The results are compared with the weight of the worms at the beginning of the test and with the control(s) and the test concentrations of sediment. Each surviving animal is removed from its holding cup, rinsed briefly in deionized water (< 5 seconds) blotted dry on a Kimwipe, and then placed onto a pre dried, pre weighed, pre labeled weigh boat. The aluminum foil boat should be folded over to prevent the loss of the animal during drying. Note weigh boats should be handled with forceps only. Oven dry animals at 60°C for 24 hours, remove animals and boats from oven and allow to come to room temperature in a desiccator prior to weighing on a microbalance to the nearest 0.01 mg. Subtract boat weight from total weight to obtain measured dry weight value of surviving worms.

Growth Modification (Ash-Free Dry Weight). The purpose of this modification is to account for the weight of sediment contained in the gut of the worms during the drying process. Worms reared under similar conditions and life history, but exposed to different grain size sediment, may express significantly different dry weights due to the contribution of heavier gut material of the worms maintained in sandy (heavier particles) sediment. This discrepancy has the potential to lead to Type II errors, where significant differences are found between test treatments, when none actually exist. The procedure below is a tool to estimate the actual contribution of gut content to the overall weight of the animals. A procedure defined as “ashing” is employed to heat the worm tissue at high temperatures until all that is left behind is solid inorganic material.

At the termination of the 20-day survival and growth test, sediment from each test chamber is sieved through a 0.5-mm screen and all recovered polychaetes are transferred into a plastic cup. Survival is recorded and worms are rinsed with deionized water and placed in pre-ashed, pre-weighed (tare weight) aluminum boats and dried in a gravimetric oven at 60°C for at least 24 hours (dry on upper shelf of oven). Each weigh-boat is removed from the oven, cooled in a desiccator for approximately 30 minutes, and then weighed on an analytical microbalance to

0.01 mg to determine total dry weight. Each of the weigh boats is then dried in a muffle furnace heated to 550°C for 2 hours, cooled, placed in dessicator for at least 30 minutes, and then weighed on an analytical microbalance to 0.01 mg to determine total ashed weights.

Endpoints are calculated as follows:

$$Dry\ weight\ biomass\ per\ replicate\ (mg) = total\ dry\ weight - tare\ weight$$

$$AFDW\ biomass\ per\ replicate\ (mg) = total\ dry\ weight - total\ ashed\ weight$$

$$Dry\ weight\ growth\ per\ individual\ (mg/individual) = \frac{total\ dry\ weight - tare\ weight}{number\ of\ survivors}$$

$$AFDW\ growth\ per\ individual\ \left(\frac{mg}{ind}\right) = \frac{total\ dry\ weight - total\ ashed\ weight}{number\ of\ survivors}$$

$$Dry\ weight\ individual\ growth\ rate\ \left(\frac{\frac{mg}{ind}}{day}\right)$$
$$= \frac{dry\ weight\ growth\ per\ individual - average\ pretest\ dry\ weight\ per\ individual}{20\ days\ of\ exposure}$$

$$AFDW\ individual\ growth\ rate\ \left(\frac{\frac{mg}{ind}}{day}\right)$$
$$= \frac{AFDW\ growth\ per\ individual - average\ pretest\ AFDW\ per\ individual}{20\ days\ of\ exposure}$$

6.3 Post-task

Results Needed:

- Percent mortality for each treatment
- Dry weight and AFDW biomass for each treatment
- Dry weight and AFDW growth per individual for each treatment
- Dry weight and AFDW individual growth rate for each treatment
- Mean/min/max water quality values by treatment, plus ammonia (total and unionized) and sulfides (total and hydrogen sulfide) for Day 0 and Day 20 porewater and overlying water
- LC_{50}, NOEC and 95% confidence limits (for ref. tox.)
- Tables showing biological, chemical, and physical data

In screening tests, the responses of worms in collected test sediments are compared to control and reference site sediments.

6.4 Reporting

The report may include, but will not be limited to, the following:

- Name and address of the laboratory conducting the study, and dates on which the study was initiated and completed.
- The name of the Project Manager, other scientists or professionals, and supervisory personnel involved in the study.
- Objectives as stated in the protocol.
- A description of the methods used.
- Transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusion drawn from the analysis.
- The test substance identified by code number and the date each sample was used.
- The number of organisms used in the study.
- Concentrations of exposure and exposure method.
- Any circumstances that may have affected the quality or integrity of the data, including deviations from test protocols or Standard Operating Procedures.
- Additions or corrections to a final report will be in the form of an amendment by the Project Manager. The amendment will clearly identify that part of the final report that is being altered and the reason(s) for the alteration(s). The amendment will be signed and dated by the Project Manager.

The master copy of the final report will be signed and dated by the Project Manager.

7.0 HEALTH AND SAFETY CONSIDERATIONS

Proper laboratory protection, including lab hood or ventilation system, lab coat, closed-toe shoes, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

Refer to the Port Gamble Laboratory's Health and Safety Plan at S:\Health and Safety for procedures to ensure safe operation in the laboratory and for contingency plans in the event of an accident or emergency.

For specific chemical health and safety information, refer to the Safety Data Sheet log.

8.0 PERSONNEL

Any laboratory personnel demonstrating competence with this method may perform the procedure.

9.0 QUALITY ASSURANCE REQUIREMENTS (ACCEPTANCE CRITERIA)

This study will be conducted according to the Standard Operating Procedures which are in effect during the time the study is being performed. In the case where there is a conflict between the other SOPs and this protocol, the protocol will be the definitive procedure.

Usually tests would be unacceptable if one or more of the following occurred:

- More than 10% of the control organisms die.
- The dry weight growth rate was < 0.38 mg/ind/day
- The initial size was not between 0.25 and 1.0 mg dry weight
- All test chambers were not identical.
- Treatments were not randomly assigned to test chambers.
- Test organisms were not randomly or impartially distributed to test chambers.
- All test animals were not from the same population, were not all of the same species, or were not of acceptable quality.
- Reference sediment and controls were not included in the test.
- Temperature, DO, pH, salinity, and ammonia were not measured, or were not within acceptable range.
- Aeration to the test chamber was off for an extended time such that the DO levels dropped to less than 4.6 mg/L.
- Response criteria were not monitored in a random fashion.

10.0 REFERENCE DOCUMENTS

10.1 Internal Documents

SOP EQP060 : Calibration/Operation/Maintenance of Orion Star Model A329 pH/ISE/Conductivity/ DO Meter (pH)
SOP EQP061: Calibration/Operation/Maintenance of Orion Star Model A329 pH/ISE/Conductivity/DO Meter (DO)
SOP EQP062: Calibration/Operation/Maintenance of Orion Star Model A329 pH/ISE/Conductivity/DO Meter (Conductivity and Temperature)
SOP LAB019: Labware Washing Procedures
SOP LAB063: Sampling and Measurement of Total Ammonia in Water
SOP LAB017: Sulfide Sampling With Ion Selective Probe
SOP LAB018: Sulfide Sampling and Measurement with Spectrophotometer

10.2 External Documents

ASTM. 2013. Guide for conducting Sediment Toxicity Test with Marine and Estuarine Polychaetous Annelids. Standard Guide #E-1611-00 (Reapproved 2013). American Society for Testing and Materials, Philadelphia, P A.

Inouye, L., E. Hoffman, D. Fox. 2015. DMMP Clarification Paper : Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing for Marine Bioassays.

Kendall, D. 1996. PSDDA/SMS Clarification Paper: Neanthes 20-Day Growth Bioassay – Further Clarification on Negative Control Growth Standard, Initial Size, and Feeding Protocol.

Kendall, D., R. McMillan, B. Gardiner, B. Hester, J.D. Word. 2013. DMMP/SMS Clarification Paper: Bioassay Endpoint Refinements: Bivalve Larval and Neanthes Growth Bioassays.

Puget Sound Water Quality Authority (PSEP). 1995. “Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments.” Prepared for U.S. EPA Region 10, Office of Puget Sound. Seattle, W A.

11.0 APPENDIX OF CHANGE

- 08/20/15 Hand-written change to reflect criteria: if >5% mortality 48hrs preceding test, organisms should be replaced

- 11/12/15 Updated logo, corrected microbalance to nearest gram, and changed test organism acclimation section to reflect a 10% mortality threshold when assessing the organism's health during rounds

- 05/23/16 Added "uncontrolled" statement to SOP footer and updated Health and Safety section

- 05/09/17 Updated health and safety information, removed branding, added review documentation section. Added "proprietary information" statement in footer. Updated information on animal health upon receipt in section 6.1.3.

- 12/1/21 Added reference toxicant section. Updated references. Added limits for ammonia and sulfides. Specified that initial weight requirements are dry weight. Added control growth acceptability criterion. Added endpoint calculations.

12.0 DOCUMENT REVIEW

SOP Approval

Name	Signature	Title	Date
		Quality Systems Director	
		Laboratory Manager	

Acknowledgement below indicates that the individuals have read and understood the concepts summarized in this document.

Name	Signature	Date

SOP Number E16
Overview of Manual Data Validation Process

STANDARD OPERATING PROCEDURE		
Title: Overview of Manual Data Validation Process		
Lead Author	**Approval**	**Document No: DVN-500**
Christina Frans, QA Manager	Alison Bodkin, Senior Chemist	**Revision No.: 4**
Signature: Christina M. Frans	Signature: Alison M. Bodkin	Revision Date: 10/30/2018
Date: 10/20/18	Date: 10/30/18	Supersedes: DVN-500, Rev. 3

1.0 SCOPE/APPLICATION

This Standard Operation Procedure (SOP) defines the process, responsibilities and documentation required by EcoChem, Inc. to perform manual data validation. This SOP is applicable to all levels of validation.

EcoChem incorporates electronic validation of quality control (QC) samples when an appropriate electronic data deliverable (EDD) is received and incorporated in the Data Quality & Usability Electronic Screening Tool (DQUEST). Separate instructions for steps involving the use of DQUEST are provided in relevant sections.

2.0 LIST OF REFERENCES AND ASSOCIATED SOP

USE THE MOST CURRENT REVISION OF THESE CONTROLLED DOCUMENTS

EcoChem Data Validation Criteria Tables

SOP DV-501, Data Validation Module A – Completeness and Holding Times

SOP DV-502, Data Validation Module B Organics – Technical Evaluation of Quality Control and Instrument Performance

SOP DV-503, Data Validation Module B Inorganic – Technical Evaluation of Quality Control and Instrument Performance

SOP DV-504, Data Validation Module C – Evaluation of Compound Identification and Quantitation

SOP DV-505, Secondary Review of Data Validation Technical Report

SOP DV-506, Project Management Report Review and Approval of Data Validation Report

SOP DV-407, Data Log-In for Data Validation Projects

SOP DV-409, Data Validation Work Flow

3.0 DEFINITIONS AND ACRONYMS

	REQUIRED ECOCHEM DOCUMENTATION MODULES			
ECOCHEM LEVEL OF REVIEW	***A***	***B (SECTION 1)***	***B (SECTION 2)***	***C***
Compliance Screening - Also referred to as: • Stages 1 & 2A – Manual and/or Electronic (EPA) • QA-1 (PSDDA/PSEP) • Cursory • Verification	X	X		
Summary Validation - Also referred to as: • Level 3 (EPA CLP) • Stage 2B – Manual and/or Electronic (EPA) • Screening (AFCEE) • M-2 (EPA Region 3) • IM-2 (inorganics EPA Region 3) • CLP summary form review	X	X	X	
Full Validation - Also referred to as: • Levels 4 and 5 (EPA CLP) • Stages 3 or 4, dependent on method/instrumentation – Manual and/or Electronic (EPA) • QA-2 (PSDDA/PSEP) • Definitive (AFCEE) • M-3 (organics EPA Region 3) • IM-3 (inorganics EPA Region 3)	X	X	X	X

A module will consist of the question and answer (Q&A) checklist, appropriate criteria tables and required supplemental worksheets.

Module A: Evaluation of package completeness, sample chain-of-custody and preservation and holding times.

Module B:

***Section 1*:** Evaluation of blank contamination; precision (replicate analyses); accuracy (compound recovery); detection limits.

***Section 2*:** Instrument performance (initial and continuing calibration, tuning, sensitivity and degradation).

Note (1): Instrument performance sections of Module B are not completed for Compliance Screening Level.

Module C: Evaluation of compound identification and quantitation (transcription and calculation checks).

4.0 DOCUMENTATION AND RECORDS

4.1 General

All worksheets and checklists shall be completed digitally and saved in the appropriate project subfolder on the network.

Note: Due to frequent edits and technical updates, the most recent worksheets, checklists and other controlled process documents, referred to in the following sections, are available on the network in the folder designated for controlled documents.

If a project requires modified documentation and/or Criteria Tables, they will also be available on the network in the folder designated for client or project specific controlled documents.

4.2 Data Validation Work Order

The Project Coordinator, Project Manager or designee, shall initiate the Data Validation Work Order. All information pertaining to the data group and EcoChem contractual requirements for the data validation project are documented on the Data Validation Work Order (see SOP DV-407). It is distributed via email to all staff assigned to the project, as well as the Project Manager, data management, etc. ('Project Team').

4.3 Data Validation Module A Checklist or DQUEST Checklist; Completeness and Holding Time

The Completeness and Holding Time Checklist documents the analytical quality control elements that have been reviewed. It must be entirely completed for each Sample Delivery Group (SDG) validated with all anomalies fully explained. The Checklist is part of the documentation required to substantiate the technical validation (see SOP DV-501). For projects utilizing the electronic screening tool (DQUEST), the DQUEST checklist must be used.

4.4 Data Validation Module B Checklist and DQUEST HRMS & ORG Tech Eval Checklists; Organics – Manual Technical Evaluation

The Module B Organics Checklist documents the analytical quality control elements that have been reviewed for organic parameters. It must be completely filled out for each Sample Delivery Group (SDG) validated, with all anomalies fully explained and all qualified data listed. The Checklist is part of the documentation required to substantiate the technical validation (see SOP DV-502).

Module B - Organics consists of two sections, Module B1 which documents the review of method precision, accuracy, blank contamination and holding time results; and Module B2 which documents the instrument specific performance results e.g. tuning, column resolution, etc. There are several Modules B2, dependent on method or instrumentation.

DQUEST Module B – Organics consists of only one section as the DQUEST Module A form documents the review of all the parameters indicated for the B1 section for non-DQUEST projects.

4.5 Data Validation Module B Checklist and DQUEST INORG Technical Evaluation Checklist; Inorganics – Manual Technical Evaluation

The Module B Inorganics Checklist documents the analytical quality control elements that have been reviewed for inorganic parameters. It must be completely filled out for each Sample Delivery Group (SDG) validated, with all anomalies fully explained and all qualified data listed. The Checklist is part of the documentation required to substantiate the technical validation (see SOP DV-503).

Module B - Inorganics consists of two sections, Module B1 that documents the review of method precision, accuracy, blank contamination and holding time results; and Module B2, which documents the instrument specific performance results e.g. tuning, calibration, etc. There is an additional Module B1 add-in for graphite furnace methods (GFAA).

DQUEST Module B – Inorganics consists of only one section as the DQUEST Module A form documents the review of all the parameters indicated for the B1 section for non-DQUEST projects.

4.6 Data Validation Module C Checklist; Calculation and Transcription Checks

The Module C Checklist documents the transcription and calculation checks that have been performed. This Checklist may include supplemental Excel spreadsheets or hand written supplemental worksheets that assist the data validation chemists in performing various calculation checks and in summarizing qualifiers that have been assigned (see SOP DV-504). There are separate Module C worksheets for organic and inorganic methods.

4.7 Secondary Validation Checklist

The Secondary Validation Checklist documents the items that are reviewed in the Secondary Review. All qualifiers are verified from the Data Validation Module Checklists to the Narrative Report and to the data results summary (see SOP DV-505).

4.8 Narrative Data Validation Report

Note: Validation chemists should never save and use draft reports on their personal drive shares or hard-drive.

A written report of the validation findings for each parameter is usually required. An electronic template shall be used. The location of the required template will be indicated by the Project

Manager and/or the Work Order. In some instances, a previous final report (from the Final Docs folder on the network) from the same client and project is used as the template.

For a *"deficiency only"* report, only quality control elements with deficiencies, qualifiers or non-compliant results will be discussed. For the *"full or all QC"* report style, in additional to discussing the deficiencies in the "deficiency only" report, each quality control element and method quality objectives (MQO) shall be summarized in the narrative report. All anomalies and problems that affect data quality or usability shall be addressed and "professional judgment" (PJ) calls documented. The narrative report may include findings from one or more SDGs, sites, sampling periods (e.g., quarterly) etc., as requested by the client.

4.9 Communication Records

Communication records are maintained electronically and may include emails, email summaries of phone conversations, and/or pdf copies of letters and faxes. Include enough details to identify the communication such as the date, client name, project or site name, EcoChem project and phase number, SDG, sample IDs, laboratory name, and person contacted. For telephone conversations that result in a decision or action, record the conversations in a summary email to the pertinent party including questions, need for re-submissions, actions and responses. Many projects require the Project Manager to be the hub for all client or laboratory communications; this should be indicated on the Project Set-up documentation.

When requested, communication records will be included as an appendix to the validation report. To facilitate inclusion of these communications with the laboratory, client or other data users in the data validation report, electronic copies in portable document format (PDF) shall be generated and saved in the project files.

4.10 Project Management Review and Approval Checklist

The Project Management Review and Approval Checklist shall be completed for each data validation report that is prepared by EcoChem. It shall be initiated by the Project Manager and approved for release by the Project Advisor. The Checklist documents all reviews performed on the report package: Final Report Review, Data Validation Qualifiers Review, and Approval for Release (See SOP-DV-506).

5.0 RESPONSIBILITIES

Responsibilities for technical data validation and internal quality control are assigned as follows.

5.1 Project Coordinator

- Log-in data, following SOP DV-407.

- CONDITIONAL: Depending on the project's complexity and workload, the Project Coordinator may be required to verify the sample and QC result summaries (laboratory report submitted as a PDF) to the EDD printout.
- Create Sample Index (manually or from laboratory electronic data deliverable (EDD).
- Notify Project Manager if any data are missing from packages or other major issues are noted during log-in.
- Perform final format review of completed validation reports after Primary validation and Secondary Review is completed.
- Assist the Project Manager, as requested including assembling and formatting the final report and/or sending final report.

5.2 Primary Validation Chemist

- If required by the project, perform EDD verification (if not assigned to Project Coordinator). If errors are found, alert the Project Manager.
- Complete Module A Checklist and apply any required qualifiers to the project database or spreadsheet.
- Perform data validation indicated on Work Order using appropriate checklists and Criteria Tables.
- Contact laboratory (if authorized on Project Set-Up) to request additional or supplementary data submissions or to discuss findings and possible corrective actions. Document laboratory/client contact in an email.
- Document, correctly and completely, all data inconsistencies, non-conformances, errors and problems on the Module checklists.
- Draft the narrative data validation report using required template and format.
- Review own work prior to Secondary Review.
- Notify the Secondary Validation Chemist that worksheets are ready for review. These include completed EDD verification, Module A, B and/or C checklists, Supplementary Worksheets, qualified sample results in the database, and draft DV report.
- Resolve any suggested changes and corrections with Secondary Validation Chemist.
- Complete validation on schedule and within assigned labor budget. Discuss all possible time and budget overruns with the Project Manager - *before they occur*.

5.3 Secondary Validation Chemist

- Review project documents e.g., completed EDD verification, Module Checklists, supplementary worksheets, qualified sample results in database, and written report. Refer to laboratory data package as needed.

- If there are major changes and edits to the report, use 'track changes' in MSWord™.
- Discuss changes and corrections with the Primary Validation Chemist.
- Document Secondary Review on the Secondary Validation Checklist.
- Submit report and folder to Project Coordinator for formatting and report compilation.
- Complete Secondary Review on schedule and within assigned labor budget. Discuss all possible time and budget overruns with the Project Manager - before they occur.

5.4 Project Manager

- Act as point-of-contact between the client and EcoChem for project technical, schedule and budgetary concerns.
- Track, schedule and budget the project.
- Communicate all project requirements, via Validation Project Set-Up, Data Validation Work Order and supplementary instructions, to technical project team including validators, secondary reviewers, data management and Project Coordinator.
- If there are major changes and edits to the report, use 'track changes' in MSWord™.
- Review final submittal for adherence to EcoChem Quality Assurance protocols and documentation process as discussed in all SOP.
- Document "approval for release" of final report on Report Quality Control and Approval Checklist.
- Insure that final report and database are transmitted on time.

6.0 PROCEDURES

6.1 Module A – Completeness, Chain-of-Custody, and Holding Times Summary

The Module A Checklist is filled out completely by the Primary Validation Chemist using the techniques described in SOP DV-501.

The Module A Checklist and supplementary worksheets (completeness check, holding time tables) are saved in the active project folder on the network.

An electronic Sample Index can be created at this time.

6.2 Module B – Summary Forms (Samples, Surrogates, Spikes, Duplicates, Blanks, Calibrations)

6.2.1 The Module B Checklist is filled out completely by the Primary Validation Chemist using the techniques described in SOP DV-502 (organics) or SOP DV-503 (inorganics). Note: Module B may contain two sections: Section B-1 includes validation of Samples, Surrogates, Spikes,

Duplicates and Blanks; Section B-2 includes validation of calibrations and instrument performance. Some methods (e.g., high resolution mass spectrometry) require a specialized Section B-2. There is a place on the Module B Checklist to indicate which level of validation is required and performed.

6.2.2 If calculation and transcription checks are not required, the Primary Validation Chemist writes the data validation report and transfers the Module A and Module B Checklists, supplemental worksheets, and qualified sample result summaries to the Secondary Review Chemist.

6.2.3 If calculation and transcription checks are required, the Primary Validation Chemist goes to Module C as described in Section 6.3.

6.3 Module C – Calculation and Transcription Checks

6.3.1 Using approved recalculation spreadsheets, perform calculation and transcription checks.

6.3.2 The Module C Checklist is filled out completely by the Primary Validation Chemist using the techniques described in SOP DV-504.

6.3.3 The Primary Validation Chemist writes the data validation report and notifies the Secondary Review Chemist that the Module A, Module B and Module C Checklists, supplemental worksheets, qualified sample result summaries, and data validation report are ready for review.

6.4 Secondary Validation

6.4.1 The Secondary Validation Checklist is filled out completely by the Secondary Review Chemist using the techniques described in SOP DV-505. The Secondary Review Chemist discusses any changes or corrections with Primary Validation Chemist.

6.5 Final Report

- The Project Coordinator formats and prepares the final report.
- The Project Manager reviews the final report, qualified data tables or summary forms, sample index, communication records, and other information for consistency and accuracy as described in SOP DV-506.
- The Project Manager also approves the report for release as described in SOP DV-506.
- The Project Manager or Project Coordinator files all final deliverables in the Final Docs folder and prepares the PDF for transmittal to the client.

6.6 Project Close

- The Project Coordinator, under the guidance of the Project Manager, should delete draft report copies from the project folder and transfer any technical documents to the Final Docs folder on the network.

SOP Number E19
Subsurface Sediment Collection

STANDARD OPERATING PROCEDURE E19

SUBSURFACE SEDIMENT COLLECTION

A Introduction

Subsurface sediment core samples will be collected primarily from a sampling vessel (using a vibracorer or drill rig), or they will be manually collected from nearshore intertidal or high-elevation areas where access from a vessel is not possible. Procedures for these two access options are described below.

B Sample Collection by Boat

B1 Collect Sediment

When sampling from a boat, most of the sediment cores will be collected using a vibracorer. Some vertical cores in areas that are difficult to sample may be collected via drill rig. The vibracorer will be deployed from the sampling vessel using an A-frame with a hydraulic winch system. The vibracorer consists of a vibrating power head attached to a 4-in.-diameter core barrel (length to be dependent on the target core depth). Where used, the drill rig will be stationed on a barge or similar floating platform. The drill rig will use rotary sonic methods to advance a core barrel to the depth of sampling. Once the sample depth is reached, an outer casing will be advanced to the same depth as the core barrel, and the core barrel will be retrieved for sample processing. Continuous sediment cores will be collected using the vibracorer or the drill rig.

Sediment core samples will be collected and processed according to the following procedures:

1. The sampling vessel will be maneuvered to the proposed sampling location using a differential global positioning system (DGPS)[1] with sub-meter accuracy, positioned such that the DGPS receiver (located on top of the sampling frame) is within 3 m (10 ft) of the target sampling location.
2. If it is not possible to access the target location due to obstructions or difficult substrate (e.g., presence of riprap or other debris), the vessel may be relocated within 10 m (32 ft) of the proposed location.

[1] A Trimble© SPS461 or similar DGPS receiver unit will be employed for the various sampling methods outlined in the quality assurance project plan. The DGPS receiver will be calibrated daily to ensure that it is accurately recording positions from known benchmarks and functioning within the individual unit's factory specifications.

3. The vibracorer with decontaminated[2] core tube or drill rig with decontaminated core barrel will be deployed.
4. Continuous core samples will be collected to the project depth requirement or until refusal.
5. The depth of core penetration will be measured and recorded.
6. The sample core tube will be extracted, and the assembly will be retrieved aboard the vessel.
7. The core sample will be evaluated at the visible ends of the core tube to verify retention of the sediment in the core tube.
8. If the sediment core is acceptable (see criteria below), the core will be capped, labelled, and held vertically pending transfer to a processing crew.
9. The top of the core will be decanted and the top of the mudline will be marked on the core tube when possible.

Acceptance criteria for a sediment core sample are as follows:

- The material is collected to the target depth within the first three attempts.
- Recovery is at least 75% of the penetration depth.
- The core appears to be intact without obstructions or blocking.

If sample acceptance criteria are not achieved, the sample will be rejected. If repeated deployment (i.e., maximum three attempts) does not result in a sample that meets the acceptance criteria, or if deployment hits refusal before reaching the target depth, the sample with the best penetration depth will be retained.

Field forms and notes for all core samples will be maintained as samples are collected. The following information will be included in the sediment core collection forms and field notes:

- Water depth and tidal elevation (i.e., raw data), as well as the calculated mudline elevation of each sediment core location relative to mean lower low water
- Location of each sediment core as determined using a differential global positioning system with sub-meter accuracy
- Date and time of collection for each sediment core
- Names of field supervisor and person(s) collecting and logging the sample
- Core penetration and recovery measurements
- Designation of each coring attempt as "accepted" or "rejected"

[2] All equipment will be decontaminated following procedures described in Section D of this appendix.

- Observations made during sample collection, including weather conditions, complications, ship traffic, and other details associated with the sampling effort
- Core type and location identification (ID)
- Photographs of anything of note
- Any deviations from the approved sampling plan (on a Protocol Modification Form [Appendix E])

B2 Process Core

Sediment cores collected from a boat will be processed as soon as possible after a core has been collected that meets the acceptance criteria. A field geologist or geotechnical engineer will oversee the sediment core logging process. The steps for processing the samples are as follows:

1. Prior to processing, evaluate any additional amount of compaction that may have occurred after core acceptance and prior to core processing, and calculate the adjusted recovery percentage (ARP) to be applied during core processing.

    - Measure the core processing recovery depth (i.e., the compacted core depth prior to processing).
    - To calculate the ARP, divide the processing recovery depth by the penetration depth (i.e., the depth recorded during core collection and acceptance).
    - Example: If the core processing recovery depth (i.e., adjusted depth) at the time of processing is 2.55 ft, and the core penetration depth (i.e., at the time of collection) was 3.00 ft, the ARP would be 85.0% (e.g., 0.85).

2. Where rotary sonic drilling is used, extrude the sample from the core barrel into a plastic liner.
3. Carefully cut along the core tube or liner to expose the sediment core for processing and photograph each core.
4. A field geologist or geotechnical engineer will examine the core for major stratigraphic boundaries and to evaluate if "native" material is present in the core. If native material is observed, then all intervals with native material will be archived. Core intervals may also be modified if native stratigraphy or significant discontinuities in stratigraphy above native material are encountered. If an interval is changed to reflect a change in geologic unit, the decision will be made in the field during core processing and documented on the sediment core processing log.

5. Record the description of each core on the sediment core processing log, including the following parameters, as appropriate, and take photographs of anything of note.

   - Core penetration depth (from the sediment core collection form)
   - Processing recovery core depth and calculated ARP
   - Adjusted sample depth interval for each sample
   - Sediment grain size description following American Society for Testing and Materials (ASTM) visual-manual classification (ASTM D2488)
   - Odor (e.g., hydrogen sulfide, petroleum)
   - Vegetation
   - Debris
   - Biological activity (e.g., detritus, shells, tubes, bioturbation, live or dead organisms)
   - Presence of oil sheen
   - Any other distinguishing characteristics or features.

6. For each core, separate the material from each target depth interval, applying (i.e., multiplying) the ARP to the target sample depth that will constitute the sample for laboratory analysis. For example, if the ARP for a subtidal sediment core is 85.0% (e.g., 0.85), the sample material to collect for a 0- to 60-cm analysis will come from the 0- to 51-cm interval (i.e., 60 cm × 0.85 = 51 cm).
7. Transfer each sediment sample into a separate stainless steel bowl for homogenization.

   - For intertidal sediment cores, the target sample depth interval is 0 to 45 cm.
   - For subtidal sediment cores, the target sample depth interval is 0 to 60 cm.
   - For shoaling areas, the target sample depth interval is dependent on the thickness of the shoaled material (see Appendix F for estimated shoal depths).
   - For vertical extent cores (i.e., cores collected to target depths that are deeper than the remedial action level [RAL] intervals described in the first three bullets of this step), sample processing will occur as follows:
     i. Samples will also be collected from 30-cm intervals beyond the RAL depth intervals for archive or analysis. Generally, the first two 30-cm intervals below the RAL interval will be analyzed, and then each subsequent alternating interval will be archived or analyzed, until reaching the end of the core, native sediment, or the target depth. Appendix F (Table F-4) identifies the analysis and archive intervals for each vertical extent core.

ii. If more than 15 cm of sediment is collected below the target depth for a given core, additional interval(s) will be archived. If there is more than 45 cm of sediment, then multiple intervals will be archived.

iii. For vertical extent cores, grain size will be analyzed in one or more composite samples representing the full length of the core above any native material layer encountered. The compositing interval(s) will be determined when the core is examined. Grain size analysis will not be needed for every core; during sediment core logging, the field geologist or geotechnical engineer will identify cores to obtain the grain size composite(s) that will be spatially representative.

- If multiple cores are collected to meet minimum volume requirements (e.g., for chemistry and toxicity analyses), the target sample intervals from all cores will be composited prior to homogenization.

8. Homogenize the sediment using clean stainless steel spoons until texture and color homogeneity have been achieved, removing large non-sediment items such as gravel, shells, wood chips, or organisms (e.g., clams) (Ecology 2017).
9. Affix a complete sample label to each individual sample jar. Sample labels will contain the project number, sampling personnel, date, time, and sample ID. Labels will be filled out as completely as possible prior to each sampling event.
10. Dispense sediment into clean and labelled jars. For any location where toxicity testing is planned, dispense samples from the bowl of homogenized sample material for both toxicity testing and (in separate jars) sulfides and ammonia analysis. These analyses will be expedited in order to have data available prior to the initiation of toxicity testing. Collect subsamples for sulfides and ammonia from the homogenized composite sample. Place each sulfide subsample in a 4-oz. jar with a Teflon® septa, filled so that there is zero headspace. The sample jar will contain 5 mL of 2 Normal zinc acetate per 30 g of sediment as a preservative. Cover the sulfide sample with zinc acetate solution in the jar using a pipette and shake vigorously to completely expose the sediment to the zinc acetate. Label the jar to indicate that zinc acetate has been added and store in the dark at 0 to 6°C.
11. Thoroughly check all sample containers for proper identification, analysis type, and lid tightness. The field coordinator will be responsible for reviewing sediment sample information recorded on field forms (Appendix E) and will correct any improperly recorded information.
12. Pack each container carefully to prevent breakage and place inside a cooler with ice for storage at the proper temperature (≤ 4 ± 2°C) for delivery to the analytical laboratory.

C Sample Collection from Shore

If an intertidal or bank sediment core cannot be collected from the boat due to site access conditions (e.g., too shallow), then the core may be manually collected from shore during a lower tide. At the discretion of the field crew, one of the following four sampling options will be used, whichever is most suitable to the sampling location conditions. In addition, the field crew may use a combined or hybrid approach of the four methods, if necessary. The bank core locations may need to be adjusted in the field to account for site conditions, such as debris or armoring, that do not allow for sampling.

C1 Option 1: Use Shovel to Dig 45-cm-deep Hole

The first sampling option is to dig a hole using a shovel and collect the sample directly from the sidewall of the hole. The process for this option is as follows.

1. **Dig hole** – Using a transplanting spade (i.e., a shovel with a narrow blade), dig a 45-cm-deep hole at the identified location. If it is not possible to reach a depth of 45 cm within three attempts, the deepest hole among the attempts will be sampled using the methodology described below, and the depth of refusal will be recorded on the sediment core collection form. At least one side of the hole should be approximately vertical to allow for the collection of the sample. Record any necessary revisions of the sampling location.

2. **Prepare for sampling** – Divide the vertical extent of the hole into three 15-cm sections (i.e., the bottom section 30 to 45 cm below the surface, the middle section 15 to 30 cm below the surface, and the top section from the surface down to 15 cm). If possible, use a spoon to draw a line in the sidewall of the hole at these breakpoints. Sample the bottom section first to ensure that the sample is collected prior to the hole filling with water.

3. **Collect and homogenize sample** – Collect the same amount of sediment from each of the three 15-cm subsections along the vertical extent of the hole; collect sufficient sediment to fill a 16-oz stainless steel measuring cup. When filling the measuring cup (as described in steps 3a through 3c), exclude any debris larger than approximately 5 mm in width. If differences pertaining to the diameter of the hole are apparent (e.g., the presence of differently colored material), the resulting sample should proportionally represent all material in the hole.

    a. Starting with the bottom section of the hole (i.e., 45 to 30 cm), use a small, clean stainless steel spoon to carefully collect an even amount of sediment from the sidewall by scraping the sidewall from the bottom of the hole to the marked 30-cm line. Fill the 16-oz measuring cup using this method, and dispense the contents into a large stainless steel bowl.

b. Repeat process in the middle section of the hole (i.e., scrape the sidewall from the 30-cm to the 15-cm line) to fill the measuring cup, and dispense the contents into the bowl containing the sediment from step 3a.

c. Repeat process in the top section of the hole (i.e., 15 cm to the surface) to again fill the measuring cup, making sure to capture the full extent of this layer, including the surface material. Dispense the contents into the bowl containing the sediment from steps 3a and 3b.

d. Homogenize the contents of the bowl with a stainless steel spoon until texture and color homogeneity have been achieved, and dispense the contents into clean and labelled jars.

The procedures for processing shore-collected cores are presented below.

C2 Option 2: Use Hand-core Tube to Collect 45-cm Core

The second sampling option is to use a hand-core tube to collect a 45-cm core, extrude the core, and then collect the sample from the interior of the core. This process for this option is as follows:

1. **Collect core** – Drive the decontaminated hand-core tube (internal diameter of 7 cm) into the sediment to a depth of 45 cm at the identified location, or as near as possible based on the substrate and debris. Cap the top of the tube and pull the core out of the sediment. If it is not possible to reach a depth of 45 cm on the first attempt, up to three attempts should be made in that area (initial attempts will be retained in the core tube or extruded onto a piece of foil). After the third attempt, sample the deepest core using the methodology described below, and record the depth of refusal on the surface sediment collection form (Appendix E). Record any necessary movement of the sampling location.

2. **Collect and homogenize sample** – Extrude the contents of the core into a pre-cleaned stainless steel bowl and homogenize with a clean stainless steel spoon until texture and color homogeneity have been achieved. Discard any debris wider than approximately 5 mm.

The procedures for processing shore-collected cores are presented below.

C3 Option 3: Use Hand-operated Coring Equipment to Collect > 45-cm Core

The third option is to use hand-operated coring equipment, such as an impact corer or hand-held vibracorer (or similar equipment), to collect a core deeper than 45 cm. An impact corer system uses an impact driver powered by an engine to deliver force to advance the corer head. A hand-held vibracoring system is similar to the vibracoring system used for collection

from a vessel, except the former is smaller and more mobile. If necessary, a portable winch with rigging powered by a direct current battery may be used to recover a driven core from its collection location. Appropriate rigging would be used to attach the winch to dock or shoreline structures available near the sampling site. The sediment collection and processing methods for this option are similar to those when vibracoring from a boat, as described in Section B.

C4 Option 4: Use Land-based Drilling Methods

The fourth option is to use land-based drilling methods to collect a vertical core. Rotary sonic drilling methods will be used with a land-based drill rig. Continuous vertical samples will be collected and extruded from the drill rig core barrel. The process for this option is as follows:

1. **Collect core** – Advance the decontaminated core barrel into the sediment to the target depth at the identified location, or as near as possible based on access, substrate, and debris. Advance the outer casing to the same depth as the core barrel. Pull the core barrel out of the sediment.

2. **Extrude core** – Extrude the sample from the core barrel into a plastic liner. Log observed lithology and notable features, as described in Section B2.

3. **Collect and homogenize sample** – Subsample the core and place sampled materials in a pre-cleaned stainless steel bowl. Homogenize materials with a clean stainless steel spoon until texture and color homogeneity have been achieved. Discard any debris wider than approximately 5 mm.

The procedures for processing shore-collected cores are presented below.

C5 Processing Cores Collected from Shore

After sediment collection and homogenization have occurred, the following steps will be completed to process the sediment cores:

1. **Record information** – Record information regarding the depth of the core, sediment characteristics (e.g., color, smell, grain size, presence of debris, etc.), and necessary revisions to the sampling location on the sediment core collection and processing forms. Take photographs of anything of note and document any deviations from the approved sampling plan on a Protocol Modification Form (Appendix E).
2. **Dispense into jars** – Affix a complete sample label to each individual sample jar. Sample labels will contain the project number, sampling personnel, date, time, and sample ID. Labels will be filled out as completely as possible prior to each sampling event. Dispense sediment into labeled sample containers.

3. **QC jars and forms** – Thoroughly check all sample containers for proper identification, analysis type, and lid tightness. The field coordinator will be responsible for reviewing sediment sample information recorded on field forms (Appendix E) and will correct any improperly recorded information.
4. **Prepare for delivery to the analytical laboratory** – Pack each container carefully to prevent breakage and place inside a cooler with ice for storage at the proper temperature (≤ 4 ± 2°C) for delivery to the analytical laboratory.

D Equipment Decontamination Procedures

All sediment sampling and homogenizing equipment, including the mixing bowl and stainless steel implements, will be decontaminated between sampling locations per Washington State Department of Ecology guidelines (2017) and the following procedures:

1. Rinse with site water and wash with a scrub brush until free of sediment.
2. Wash with phosphate-free detergent.
3. Rinse with site water.
4. Rinse with distilled water.

Acid or solvent washes will not be used in the field because of safety considerations and problems associated with rinsate disposal and sample integrity, specifically:

- Use of acids or organic solvents may pose a safety hazard to the field crew.
- Disposal and spillage of acids and solvents during field activities pose an environmental concern.
- Residues of solvents and acids on sampling equipment may affect sample integrity for chemical testing.

Any sampling equipment that cannot be cleaned to the satisfaction of the field coordinator will not be used for further sampling activities.

E References

Ecology. 2017. Sediment cleanup user's manual II. Guidance for implementing the cleanup provisions of the sediment management standards, Chapter 173-204 WAC. Draft for review and comment through July 7, 2017. Pub. No. 12-09-057. Revised April 2017. Toxics Cleanup Program, Washington State Department of Ecology, Olympia, WA.