



GASTINEAU CHANNEL DISPOSAL SITE. BOUNDARIES SHOWN APPROXIMATE

A	58° 16' 45" N
B	134° 22' 54" W
C	58° 16' 41" N
D	134° 22' 46" W
E	58° 16' 42" N
F	134° 22' 58" W
G	58° 16' 38" N
H	134° 22' 50" W

GASTINEAU CHANNEL

DOUGLAS HARBOR

SANDY BEACH

UPPER CYANIDE TAILING PILE

LOWER CYANIDE TAILING PILE

TREADWELL MINE

READY BULLION MINE

APPROXIMATE MINE BOUNDARY, TYP.

GLORY HOLE

MEXICAN MINE

700-FOOT MINE



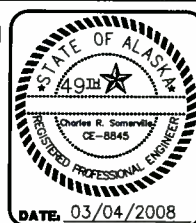
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SCALE: SCALE IN FEET
0 500 1000 FT



OLD DOUGLAS HARBOR REPLACEMENT
CBJ PROJECT NO. DH 07-133

SHEET TITLE: **DOUGLAS HARBOR/TREADWELL MINE COMPLEX/GASTINEAU CHANNEL AERIAL**

DATE: 03/04/2008

PNAD PROJECT NO. 062065.01 DWG. FILE:

4
SHEET 4 OF 4

Wanstall, Bruce (DEC)

To: Dietrick, Larry V (DEC)
Cc: Bainbridge, Steven T (DEC); Janes, William B (DEC); Sundet, Richard L (DEC)
Subject: Treadwell Mine Complex
Attachments: PND Harbor and S. Douglas Shoreline graphic.pdf; Douglas Harbor Report1.pdf

Greetings,

The public in Douglas is aware of the following argument and so should you be. If the CBJ is not aware they should be.

Attached is a graphic and the Report that provides information on the Douglas harbor sediments. The mercury layer in the sediments beneath the harbor did not occur naturally. The source of the mercury is from historic gold ore processing at Treadwell Complex mine operations along the shoreline south of the town of Douglas.

The implications to the Treadwell Mine Complex Contaminated Site is that no mill operations took place adjacent to the Douglas Harbor. The attached graphic shows each mill location and where mercury-laden tailings are now on the beach opposite the mine operation. All are located to the south of the Douglas Harbor. This means that the mercury laden tailings are not static; they are moving ever so slowly north up Gastineau Channel.

A long-shore current is an ocean current that moves parallel to shore. It is caused by swells sweeping into the shoreline at an angle and pushing water down the length of the beach in one direction. Longs-shore currents usually extend from the shallow waters inside the breaking waves to the outside breakers. They vary depending on the size, strength, and direction of the approaching swell, and the length of the beach. The more prominent the swell size and direction, and the longer and straighter the beach is, the more powerful and swift the long-shore current will be.

So, we have a situation nothing like the pulp mills in Ward Cove and Silver Bay where a blanket of new sediment will continue to bury contaminants; these contaminated tailings may be on the move and could someday present a risk of exposure.

Bruce

Bruce Wanstall

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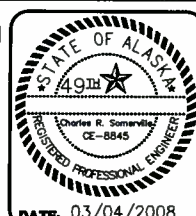
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4
SHEET 4 OF 4

Dredged Material Evaluation for the Douglas Harbor Marina Juneau, Alaska

Final Report

PREPARED FOR: PND Engineering

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March 2009



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ACRONYMS

ARI	Analytical Resources Incorporated
ASTM	American Society for Testing and Materials
BP	bioaccumulation potential
CPT	cone penetration test
COC	chain of custody
DGPS	differential global positioning system
ERED	Environmental Residue-Effects Database
ERL	effects range-low
ERM	effects range-medium
ICP-MS	inductively coupled plasma emissions spectrometer equipped with a mass detector
ID	identification
ITM	Inland Testing Manual
LPC	limiting permissible concentration
MLLW	mean lower low water
POC	point of contact
QA/QC	quality assurance/quality control
QAP	quality assurance plan
SAP	sampling and analysis plan
SIM	selective ion method
SM	Standard Methods
SOP	standard operating procedure
SP	solid phase
STFATE	Short Term Fate model
TOC	total organic carbon
USACE	United States Army Corps of Engineers
USEPA	United States Environmental Protection Agency
NewFields	NewFields Northwest LLC.

UNITS OF MEASUREMENT

°C	degree(s) Celsius
ft	feet
µg/kg	microgram(s) per kilogram
µg/L	microgram(s) per liter
µm	micrometer(s)
ng/kg	nanogram(s) per kilogram
cm	centimeter(s)
L	liter
m	meter(s)
mg/kg	milligram(s) per kilogram
mL	milliliter(s)
mm	millimeter(s)
ppb	parts per billion
ppm	parts per million
ppt	parts per thousand
v/v	volume per volume
CY	cubic yards

1 INTRODUCTION

Douglas Harbor (Figure 1-1), located in Juneau Alaska, is undergoing expansion to accommodate increased moorage demands. The expansion involves removal of existing moorings, creosote pilings, and dredged material to return the harbor to its original design depth of -14 ft MLLW. The dredging aspect of the project involves the removal and disposal of approximately 30,000 cy of sediment.

PND Engineering conducted a chemical assessment of Douglas Harbor in March 2007 (Figure 1-2). Several of the samples (PND07- 13, 14, 15, and 16) were collected in the New Harbor Dredge Area and the New Surface Dredge Areas. The concentrations of mercury detected in all of the individual sediment samples and the sediment composites were above the project screening level of 0.41 mg/kg. Five of the seven composites had mercury concentrations detected above the Puget Sound Dredged Disposal Analysis Users Manual (PSDDA) maximum level of 2.1 mg/kg. The mercury concentrations were consistent throughout the entire harbor. Mercury was the only contaminant above regulatory guidance values. Biological testing was not conducted at that time.

The current project in Douglas Harbor was designed to *verify* the concentrations of mercury present in the sediment and determine if mercury concentrations in the sediment are either toxic or bioavailable to selected species of aquatic life.

The State of Alaska does not currently have a dredged material evaluation program, therefore, federal guidance provided in the Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Inland Testing Manual (ITM; USEPA/ (USACE1998) was used to conduct field sampling and laboratory testing. The results of this study should facilitate the determination of suitability of Douglas Harbor sediment for aquatic disposal at the Gastineau Channel Dredged Material Disposal Site.

The *confirmatory* chemistry and performance of biological and bioaccumulation testing of the sediment within Douglas Harbor is a Tier III evaluation with some Tier IV assessment of the bioavailability of mercury toxicity and bioaccumulation. The results of the chemical and biological analysis were evaluated according to performance criteria outlined in the ITM (USEPA/USACE 1998) and also, when applicable, the Puget Sound Dredged Material Evaluation and Disposal Procedures (Users Manual – July 2008).

1.1 BACKGROUND AND HISTORY

Douglas Harbor has undergone a number of renovations, investigations, and dredging operations since the 1940's. The last dredging program occurred in 2003, at that time dredged material was placed in the Gastineau Channel disposal site. A summary of activities related to Douglas Harbor includes:

- 1940's: Rock fill material was placed from Douglas Island to create a street out to the City wharf near the harbor entrance.
- 1948: Juneau Island Causeway was constructed along the south margin of the basin to provide vehicle access between the mining facility and Douglas Island.
- 1961: US Army Corp of Engineers (USACE) conducted site investigations for the proposed dredging of the harbor basin and for wave protection at the entrance to the harbor.

- 1962: Harbor basin was dredged to -12 ft MLLW and an entrance breakwater was constructed. Dredged material was placed on the Douglas Island side of a containment berm located along the western limits of the basin. The placement of dredge material provided a foundation for the roadways, parks, and recreational areas known today as Savikko Park.
- 1962-65: Inner harbor facilities were designed and constructed by the State of Alaska. They included Floats A, B & C, an access dock and gangway at Float B, a tidal grid and a boat ramp.
- 1995: US ACOE Civil Works conducted Tier II sampling of the harbor basin in preparation of maintenance dredging (USACE 1995).
- 1997: The US ACOE dredged approximately 25,000 cy of material in the entrance channel and northern areas of the basin. Dredged material was disposed in an unconfined manner just outside the harbor in Gastineau Channel, an inland waterway.
- 1998: The City and Borough of Juneau (CBJ) constructed seven stall floats along the north side of Float C.
- 2001-03: The CBJ expanded the Douglas Harbor basin and installed Floats D&E resulting in the current configuration. Approximately 65,000 cy of material was dredged during this effort. A majority of the dredged material (roughly 90%) was disposed behind a geotextile lined containment berm on-site creating a boat launch ramp and parking area. The remaining dredged material was disposed in an unconfined manner outside the harbor in Gastineau Channel.
- 2007-08: The CBJ is currently planning to renovate the original section of Douglas Harbor constructed during the period 1962-65. The existing harbor facilities are severely deteriorated and need to be replaced to provide safe public moorage. The current harbor basin elevation has risen, likely due to glacial rebound and dredging is necessary to maintain safe navigational depth for vessels moored in the harbor.

The 2007 PND field survey conducted sediment sampling and physical characterization combined with chemistry analyses of the following parameters and chemicals of potential ecological concern:

- Grain size
- Total volatile Solids
- Gasoline Range Organics, Diesel Range Organics, Residual Range Organics
- Benzene, Toluene, Ethylene, and Xylene
- Polynuclear Aromatic Hydrocarbons (PAH)
- Metals
- Chlorinated hydrocarbons
- Organotins

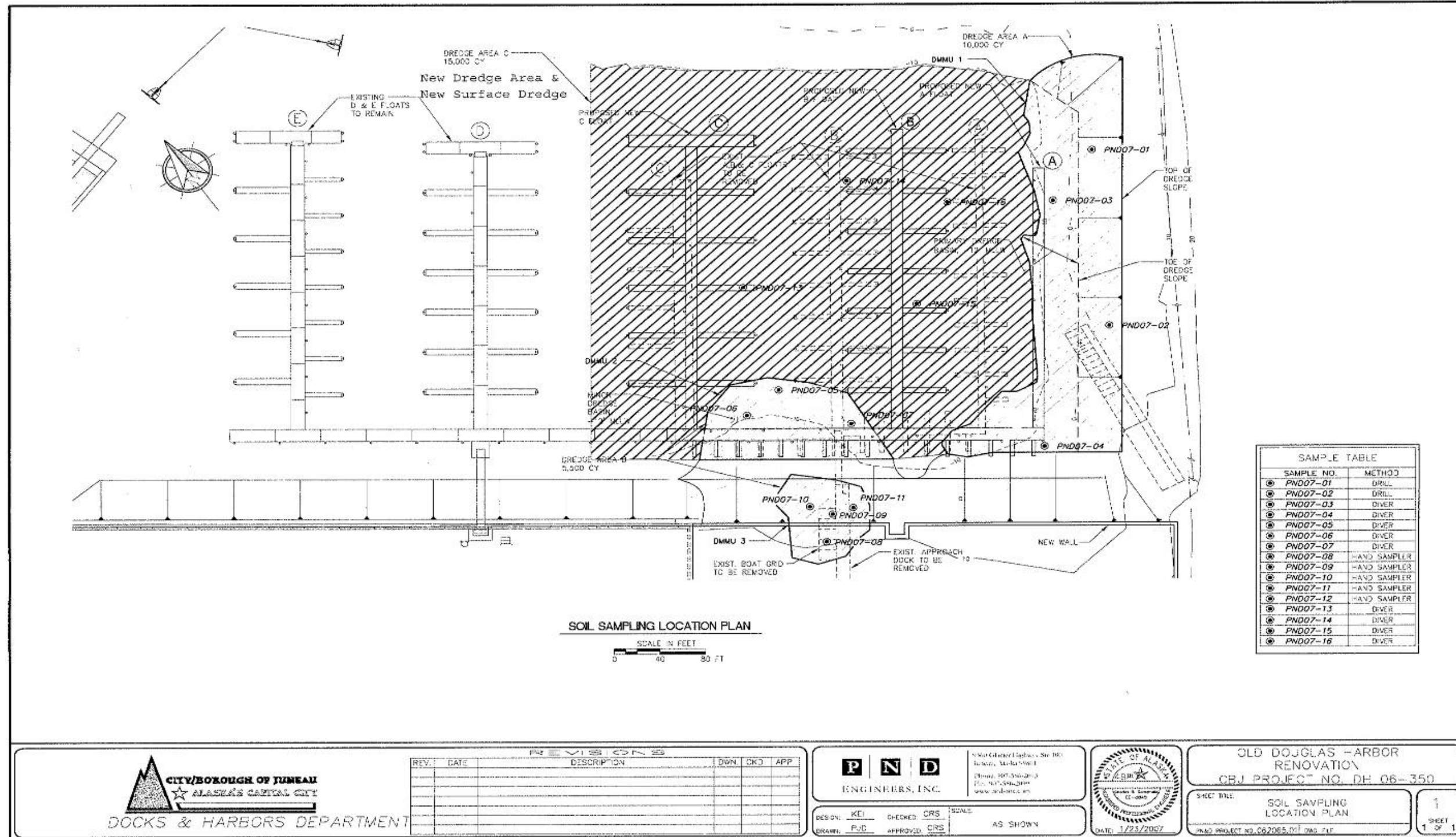


Figure 1-1. Douglas Harbor Site Map from 2007 Field Survey.

Mercury was the *only* contaminant determined to be of potential ecological concern with concentrations above the project screening level of 0.41 mg/kg and the PSDDA maximum level of 2.1 mg/kg. Mercury concentrations in the test *composites* from the 2007 survey are summarized in Table 1-1 (PND 2007; Data taken from PND Report #062065, p10). Individual sediment sample concentrations ranged from 0.47 to 5.4 mg/kg.

Table 1-1. Mercury Concentrations in Composite Sediment Samples, 2007.

Sample Location	Mercury Concentration (mg/kg dry weight)
PND-11	1.3
PND-2	2.4
PND-4	2.5
Harbor Dredge	3.5
New Surface Dredge	2.2
PND-1	1.8
PND-3	2.7

Concentrations of the other potential contaminants of concern were below screening levels and were not be analyzed as part of this program.

1.2 SAMPLING STRATEGY AND TESTING OBJECTIVES

The main objective of the current project was to verify mercury concentrations in the proposed dredged material from Douglas Harbor and to determine suitability for aquatic disposal using guidelines established in the ITM (USEPA/USACE 1998). The testing strategy paralleled the tiered testing approach (Section 3) of the ITM.

Specific project objectives were to:

- Collect test sediment to project depth using a vibratory or push core.
- Collect reference sediment from the proposed reference area (five spatial replicates and one reference composite made from five spatial replicates) using a van Veen grab.
- Conduct toxicity testing of test, reference, and control sediments using ITM methods for water-column toxicity, benthic toxicity, and bioaccumulation potential.
- Measure mercury concentrations in sediment, pore water, and tissue.
- Prepare a detailed interpretative report that includes methods, results, and a comparison of test and reference materials using ITM guidance for test acceptability and performance criteria.

Detailed sediment chemistry analysis for a variety of potential contaminants of concern was performed in 2007 as part of the Tier II assessment. The concentrations of mercury were above project screening levels; therefore this Tier III evaluation included quantification of the mercury

concentrations along with biological and bioaccumulation testing. Figure 1-2 illustrates the tiered testing approach used for this study, (figure taken directly from the ITM (USEPA/USACE 1998).

The proposed site for receipt of dredged material from Douglas Harbor is the Gastineau Channel (GC) disposal site. To determine suitability of Douglas Harbor material for disposal at this site, chemical and biological analysis included a control for test validation and reference area samples collected and tested concurrently with the test sediment following ITM procedures.

The native control sediment was specific to each type of toxicity test and species and was either collected from places where the test organisms naturally reside or was taken from cultures of test organisms in the laboratory. The response of the test organism to this sediment was used to confirm the health of the test animals and to validate the acceptability of the tests performed.

The purpose of reference sediment was to provide a point of comparison (reference point) to which benthic effects of dredged material were compared. Reference sediment was collected *outside* the influence of previous disposal operations at a dredged material disposal site, but near enough to the disposal site that the reference sediment is subject to all the same natural influences as the disposal site (USEPA/USACE 1998).

A designated reference site for the purposes of dredged material evaluation does not exist in Juneau, Alaska area. PND and the regulatory agencies (Figure 1-3) chose five different locations to represent the reference area. The five locations were tested separately and as part of a reference composite made from the five locations. There is a possibility that sediment previously disposed of at the Gastineau Channel (Figure 1-4) may have migrated outside the disposal site, therefore, the location of the reference area was placed outside of the area possibly influenced by previous disposal operations.

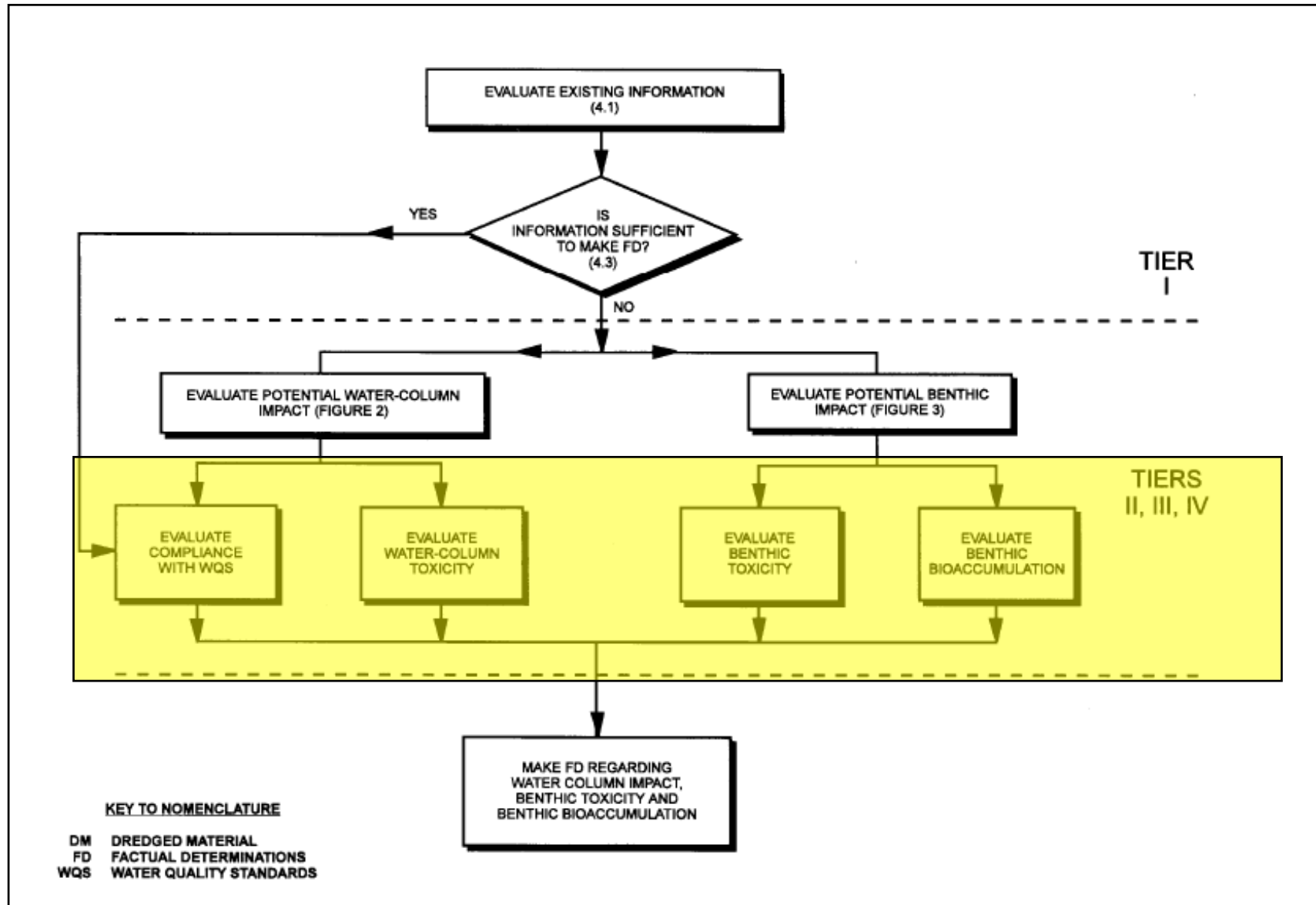


Figure 1-2. Tiered Testing Approach (ITM 1998)

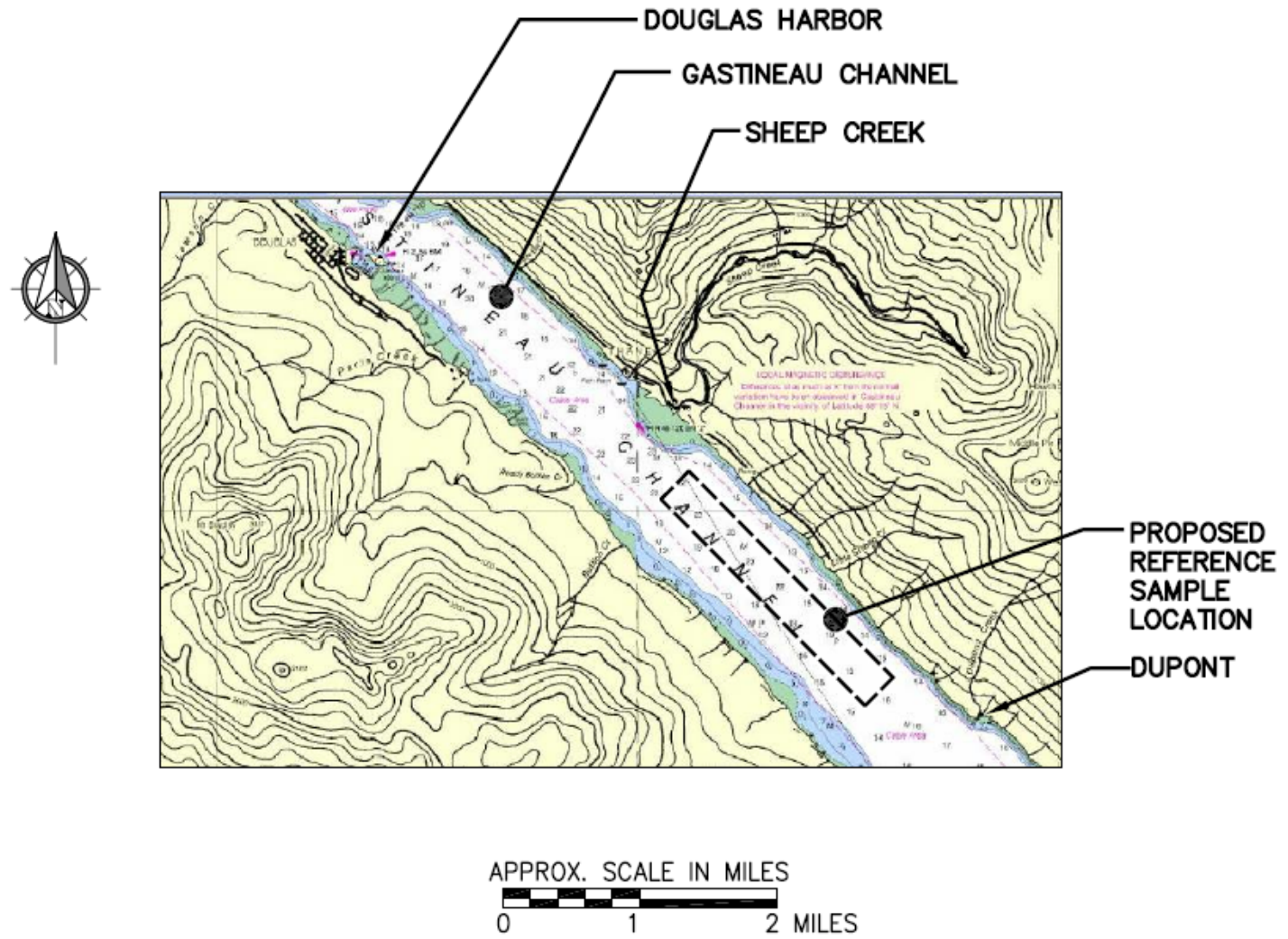


Figure 1-3. Nautical Chart of Reference Area

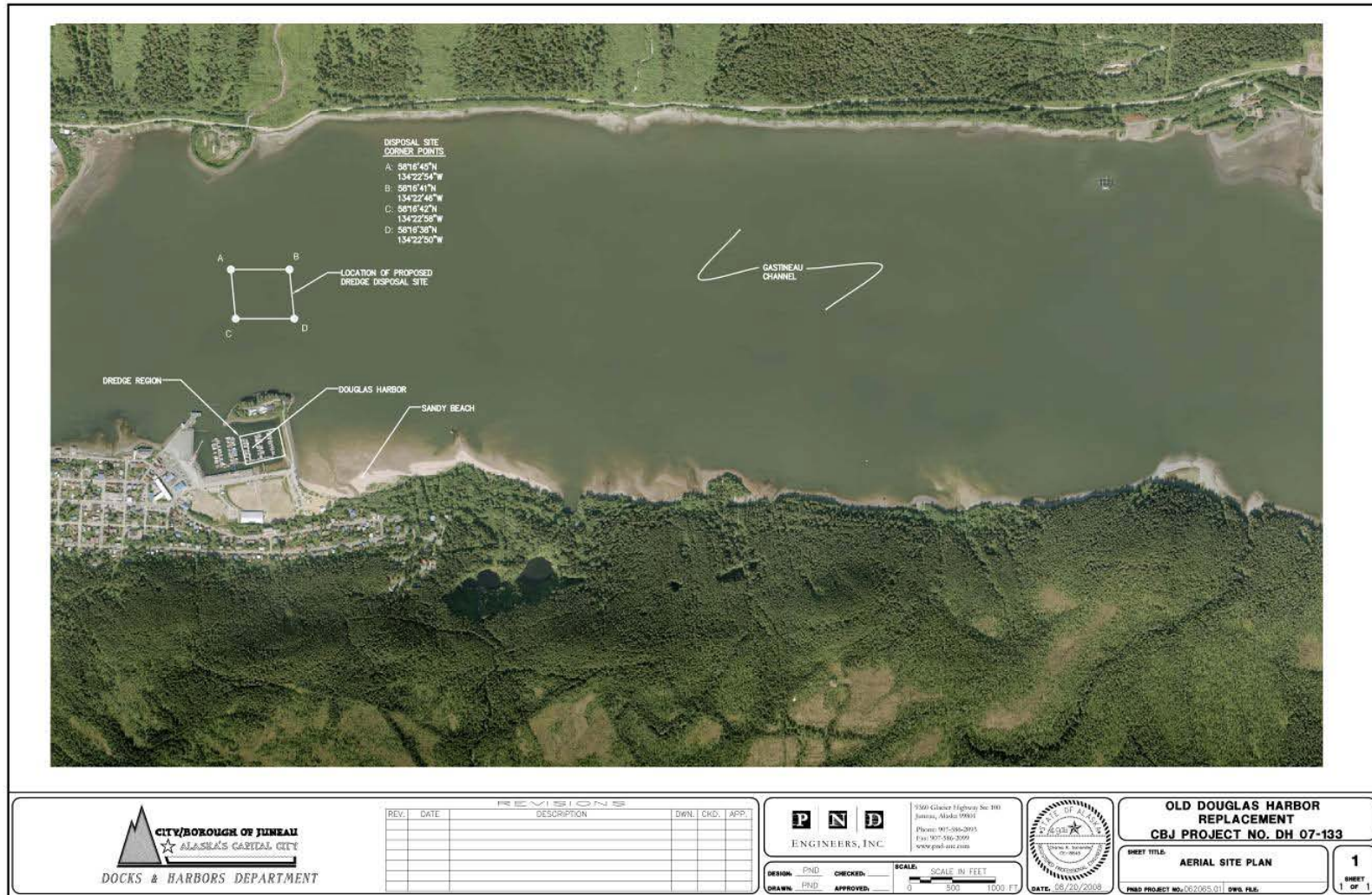


Figure 1-4. Aerial View of Douglas Harbor and the Proposed Disposal Site. *The reference area is not shown on this map.*

The five reference samples were treated as individual spatial replicates for biological testing and were submitted as individual samples for chemical analysis. These five reference samples were tested concurrently with the Douglas Harbor sediment treatments and the biological results were statistically compared to the test sediments. The comparison of reference and test sediment data provided a framework for determining suitability of the Douglas Harbor sediment for disposal at the GC site. Using the five spatial replicates in the comparison incorporates the inherent natural variability of the channel.

The five reference samples were also combined into one reference area composite based on guidance provided in the ITM when the disposal site is considered heterogeneous in nature (field investigation of the disposal site confirmed heterogeneity of disposal site, data provided in Appendix A to this report). This reference area approach is “used when the disposal site is known to be heterogeneous and more than one reference location must be sampled to adequately characterize the disposal site”.

1.3 STRATEGY FOR TESTING COMPOSITES AND STATION LOCATIONS

The estimated volume of Douglas Harbor dredged material is approximately 30,000 cy. Based on the project footprint, four area composites plus one lower composite were prepared and submitted for toxicological testing (Figure 1-5). This compositing scheme is consistent and more frequent than guidance provided in the ITM requiring a minimum of two sediment composites from eight sampling locations for volumes of 20,000-100,000 cy). The previous sediment investigation of Douglas Harbor identified four different dredged material management units (DMMU; the smallest volume of dredged material capable of being dredged independently from adjacent sediments) (PND 2007). Three of these DMMU areas (1, 2, and 4) are part of this investigation (for comparison to 2007 data the sample location names have not been changed and are shown in Figure 1-5). The sampling locations included the areas previous sampled in 2007 and a few new stations (NF prefix) to refine areas where sediment is currently accumulating.

Table 1-2. Number of Samples and Number of Composites per Dredge Volume.

Dredge Volume (cubic yards)	Number of Sampling Stations	Number of Composites
Recommended by ITM (USEPA/USACE 1998)		
5,000 – 20,000	4	1
20,000 – 100,000	8	2
100,000 – 200,000	12	3
Compositing Scheme for Douglas Harbor		
30,000	18	4

The sediment cores were opened and visually characterized prior to compositing. During this process, a change in the sediment type was observed based on depth of core with silty material in the upper layers and sandy material in the lower layers of each core. Vertical compositing was done to separate the upper and lower layers. Upper composites were kept distinct by area as designated on Figure 1-5, the lower composite represented the sandy material throughout the dredge footprint.

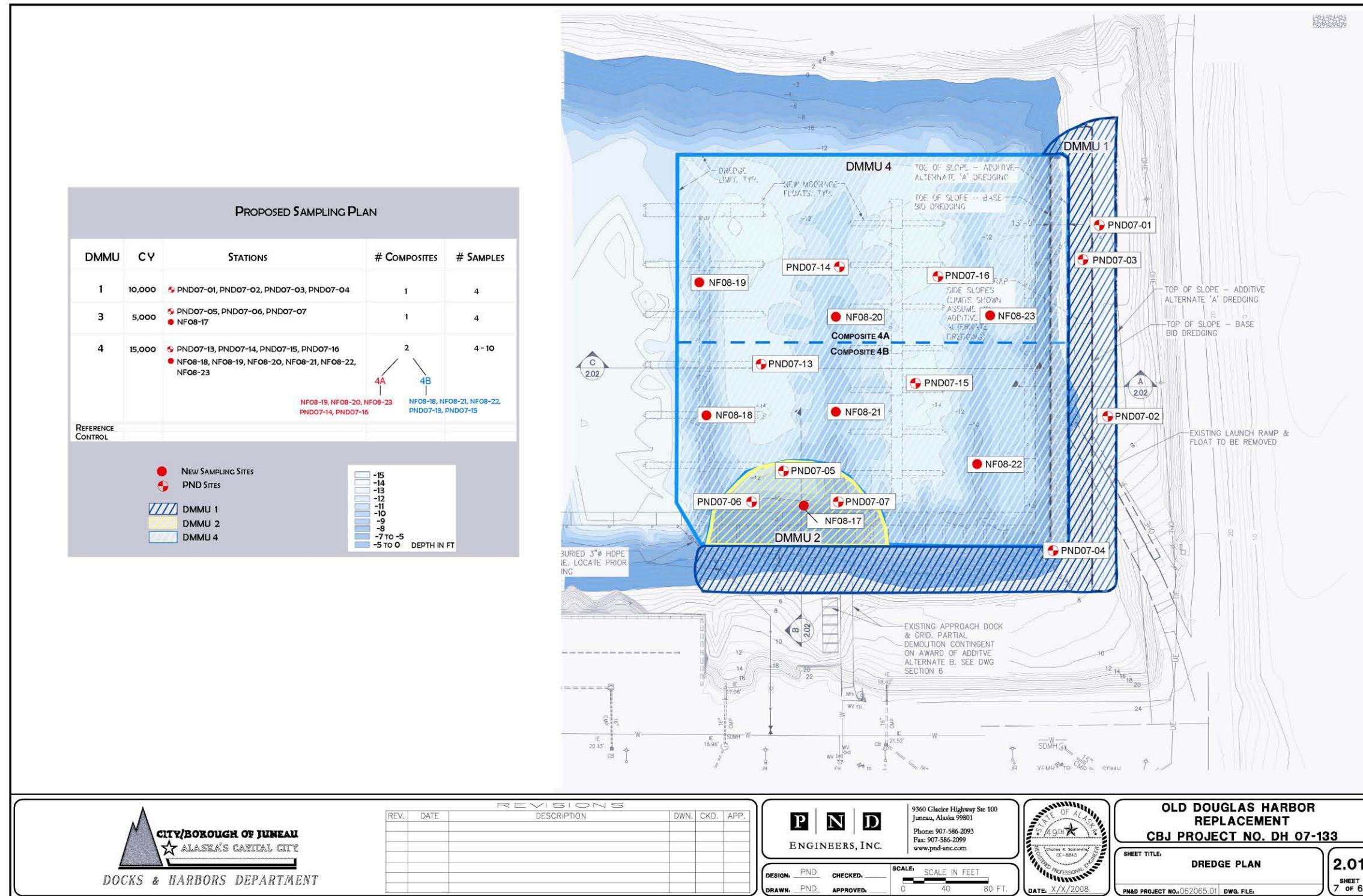


Figure 1-5. Douglas Harbor Site Map with Field Sampling Locations and Compositing Strategy.

The ITM (USEPA/USACE 1998) and the ITM Supplement (USEPA et al. 2001) provided guidance on compositing strategies:

- Combining locations from contiguous portions of the project area, using similar sediment types exposed to the same influences and pollutant sources.
- The amount of material taken from individual cores for allocation to the test composite was directly proportional to the length of core collected. The amount of test material required for each test composite (including sediment chemistry biological testing and bioaccumulation testing) was approximately ten gallons.
- The procedure for compositing included sediment from the entire length of core to project depth, however, because individual core samples contained distinct layers the core was split vertically to separate any effects that might occur from differing sediment types.

2 METHODS

2.1 FIELD SAMPLING AND SAMPLE COMPOSITING

Sediment cores were collected at eighteen stations; attempts were made to sample to a project depth of -14 ft MLLW at all locations. Table 3-1 located in the results section provides a summary of the data collected in the field.

Two sampling devices, a push core and a vibratory core, were used for the in-harbor sampling based on their ability to work in a variety of sediment types and water depths. These samplers were selected because they can collect the large sediment volumes necessary to accommodate both chemical and biological analyses.

A reference area approach was used for determination of suitability of the material for disposal. Individual reference sediment samples were collected from areas expected to be outside of the influence of the disposal site using a van Veen grab sampler. The exact locations of the reference sites were chosen in consultation with the regulatory agencies, PND, CBJ, and NewFields. The reference area samples and the reference area composite serve as a point of statistical comparison to the test data.

In addition to the reference area samples, van Veen grab samples were collected from several locations within the Gastineau Channel disposal site for characterization of the sediment type within the site.

2.1.1 CORE COLLECTION TECHNIQUES

All core sampling occurred onboard the tug vessel *WALDO* that has deck space and crane lifting capabilities to accommodate the field collection equipment. Figure 2-1 is a photograph of the vessel used for the inner harbor samples.



Figure 2-1. Waldo Vessel used for Sampling

The process for sediment collection was similar using either the push core or the vibratory hammer core except that the push core was manually pushed through the sediment and the vibratory core was vibrated through the sediment using a vibrating head. The procedure involved lowering the coring device to the sediment surface and then driving the core through the sediment to project depth. When the sampler could not penetrate to project depth due to the sediment type, the vessel was moved and a second attempt was made to collect a sample. Individual Lexan[®] core liners were used inside the core tube with separate liners used for each station. Once onboard the vessel, the core was placed horizontally on the deck and the core liner was extruded, cut into smaller sections, capped on either end, and placed in coolers containing blue ice to provide storage at temperatures of approximately 4°C.

2.1.2 VAN VEEN GRAB COLLECTION

A stainless steel van Veen grab sampler was used to collect the reference sediment samples. The R/V Summer King was used for transit to the proposed Gastineau Channel reference area. Sediment representing the upper 10 - 12 centimeters within a sampling area of 0.1 square meters was collected and transferred to labeled polyethylene bags and stored in coolers maintained at approximately 4°C during all aspects of shipping and handling. Approximately five gallons of sediment per station sample was taken, which required two to five grabs samples per site.

2.1.3 WATER COLLECTION FOR WATER COLUMN TEST PREPARATION

Douglas Harbor site water was collected into pre-cleaned polycarbonate carboys. A clean, hand operated piston type pump, was placed below the water surface and water was pumped into the clean carboy. This procedure avoids collecting any surface water that may contain oil or other materials that could interfere with the test. Approximately 120 L of site water was collected to conduct the three water column tests using a clean water pump submerged just below the water surface inside the harbor.

2.1.4 NAVIGATION

All station locations were determined using a Differential Global Positioning System (DGPS). The system uses U.S. Coast Guard differential correction data, and is accurate to ± 3 meters. All final station locations were recorded in the field using positions from the DGPS.

2.1.5 SEDIMENT HANDLING

The core stratigraphy was recorded in the field log by viewing through the clear Lexan[®] core liner. The core was cut into two to three foot sections and placed into labeled coolers maintained at approximately 4° C until delivery to the NewFields' laboratory in Port Gamble, Washington for processing. Upon return to NewFields in Port Gamble, a representative core from each station was photographed and characterized for sediment characteristics. The geologic description of each core included the texture, odor, color, length, approximate grain size distribution, and any evident stratification of the sediment. All field sampling and core processing data are summarized in Appendix A.

When the sediment cores were composed of different sediment types they were segregated into different vertical composites. The upper composites were representative of the four DMMUs discussed in Section 1.3, the lower portion of the cores were mixed into one composite representing the entire dredge footprint. Adequate sediment was collected to perform additional chemical and biological analysis, if necessary.

Sediment collected from the reference sites was placed into clean, polyethylene bags, labeled (project name, date, sampler ID), logged into a field chain-of-custody (COC) form, and placed into a cooler maintained at approximately 4° C until delivery to the NewFields' laboratory in Port Gamble, Washington for processing.

Every cooler contained a temperature blank that is used to assess the temperature of the cooler upon arrival at the testing laboratory and a chain of custody form was attached to the inside of the cooler lid.

2.1.6 SAMPLE PROCESSING AND STORAGE

Sample processing and composting was performed at the Port Gamble NewFields laboratory. Each sediment sample was homogenized to a uniform consistency at the laboratory using a stainless steel mixing bowl and spoon. Each test composite was generated by allocating sediment from each station based on the length of core collected.

Samples for physical and chemical analysis were placed into certified clean glass jars with Teflon-lined lids and shipped to the analytical laboratories. Sub-samples for archive were placed in certified clean glass jars with Teflon-lined lids and frozen at -20°C for possible future chemical analysis in the event that further delineation of chemical contamination among stations is required. The remainder of the composite sample was analyzed for toxicity and bioaccumulation potential. All sediment samples were stored in the walk-in cold room at the Port Gamble laboratory maintained at a constant temperature of approximately 4°C.

2.1.7 SHIPPING

Chemistry jars for mercury analysis were provided by the analytical laboratory (Battelle Marine Sciences Laboratory). The analysis jars were cleaned according to methods outlined for mercury analysis. Briefly, the cleaning process involved washing the bottles or glass jars and then boiling them in concentrated HNO₃ for 48 hours. Bottles were then rinsed in tap water shown to contain negligible concentrations of methyl mercury, and then filled with 0.5% HCl in low Hg water and heated to 65°C for a minimum of 24 hours. This solution was then poured off and the bottles were refilled with 0.5% HCl in low Hg water, and then stored until use. Prior to use, the vessels were emptied and dried in a clean drying oven at 65°C.

After the sediment was composited and sampled for chemical analysis, the chemistry sample jars were placed in sealable plastic bags and securely packed inside a cooler with blue ice. The COC forms were completed and the original signed COC forms were placed in a sealable plastic bag and placed inside the cooler. The cooler lids were securely taped shut.

2.2 DECONTAMINATION OF FIELD AND LABORATORY EQUIPMENT

All sampling and laboratory equipment were cleaned prior to sampling. In the field the core and grab samplers were rinsed between stations with site water. To avoid cross contamination between stations, individual core Lexan[®] liners were used to collect the sediment samples.

Sediment composting was conducted at the Port Gamble laboratory using clean sampling techniques. All stainless steel utensils (bowls, spoons, spatulas, mixers, and other utensils) were cleaned with soapy water, rinsed with tap water, and then rinsed three times with deionized water. The final cleaning step was a rinse with acetone to remove any trace of soap or organic residue. Glassware was cleaned with soapy water, rinsed with deionized water, soaked in a hydrochloric

acid bath and rinsed with acetone prior to use. After the acetone rinse the item was rinsed in deionized water again.

2.3 DOCUMENTATION AND CHAIN OF CUSTODY

Samples were considered to be in custody if they were: (1) in the custodian's possession or view, (2) retained in a secured place (under lock) with restricted access, or (3) placed in a secured container. The principal documents used to identify samples and to document possession were COC records, field logbooks, and field tracking forms. COC procedures were used for all samples throughout the collection, transport, and analytical process, and for all data and data documentation, whether in hard copy or electronic format.

The COC procedures began during sample collection. A COC record was prepared for each sample. Each person who had custody of the samples signed the form and ensured that the samples were properly secured. Minimum documentation of sample handling and custody included the following:

- Sample identification
- Sample collection date and time
- Any special notations on sample characteristics
- Initials of the person collecting the sample
- Date the sample was sent to the laboratory
- Shipping company and waybill information

2.4 PHYSICAL AND CHEMICAL ANALYSIS

Physical and chemical parameters measured in sediment for this testing program were selected to provide confirmatory data on potential chemicals of concern in the dredged material from Douglas Harbor in accordance with the ITM (USEPA/USACE 1998). Test and reference sediments were analyzed for the parameters and target detection limits indicated in Table 2-2. All analytical methods used to obtain contaminant concentrations followed EPA or Standard Methods.

2.4.1 PHYSICAL ANALYSES

To characterize the physical properties of the sediment, tests were performed to predict the behavior of sediment after disposal and to compare reference and test sediment. Physical-chemical analyses of the sediment included grain size, total organic carbon (TOC), and total solids. Grain size determines the general size classes that make up the sediment (e.g., gravel, sand, silt, and clay). The frequency distributions of the size classes (reported in millimeters [mm]) of the sediment are reported in Appendix B.

Grain size was conducted using the gravimetric procedure described in Plumb (1981). Total organic carbon (TOC), made up of volatile and nonvolatile organic compounds, was determined as recommended in the ITM (USEPA/USACE 1998) or equivalent (modified SW846). This procedure involved dissolving inorganic carbon (carbonates and bicarbonates) with hydrochloric acid or sulfuric acid prior to TOC analysis (Plumb 1981). Total solids were measured to convert concentrations of the chemical parameters from a wet-weight to a dry-weight basis. Percent solid measurements were determined by USEPA Method 160.3 (USEPA 2001).

Acid Volatile Sulfide (AVS) and Simultaneously Extracted Metals (SEM) in sediment followed the published procedure (Allen et al. 1991) for the analysis of acid volatile sulfide (AVS) in sediment

and total sulfide in aqueous samples. For sediment samples, sulfide was volatilized after the addition of acid. The acid extraction produced in this step was also analyzed for simultaneously extractable metals (SEM) that became soluble during the acidification step. As a precipitant with heavy metals, sulfide is fundamental in the determination of the bioavailability of metals in anoxic sediment. When the molar ratio of SEM to AVS exceeds one, the metals are potentially bioavailable to aquatic organisms.

Table 2-1. Physical and Chemical Measurements, Analytical Methods, and Detection Limits

Parameter	Method	Procedure	Sediment Reporting Limit (dry weight)	Water Reporting Limit	Tissue Reporting Limit (wet weight)
Grain Size	Plumb (1981)	Sieve/Pipette	1.0%		
Total Organic Carbon	ASTM D2579	Combustion IR	0.1%		
Percent Solids	EPA 160.3	Gravimetric	0.1%		
AVS/SEM	Allen et al 1991	ICP-MS	AVS: 0.0119 $\mu\text{mole/g}$ Cd: 0.0000661 $\mu\text{mole/g}$ Cu: 0.00257 $\mu\text{mole/g}$ Ni: 0.000512 $\mu\text{mole/g}$ Pb: 0.0000359 $\mu\text{mole/g}$ Zn: 0.000795 $\mu\text{mole/g}$ Hg: 0.000000278 $\mu\text{mole/g}$		
Ammonia	Standard Methods 4500 NH ₃ D ;ASTM Method D 1426-93 Test Method B; and USEPA Method 350.3	Ion Selective Method		0.5 mg/L	
Lipids	Bligh Dyer	Gravimetric			0.1%
Total Mercury (Hg) sediment and tissue	USEPA 7473	CVAA	0.002 $\mu\text{g/g}$		0.002 $\mu\text{g/g}$
Total Mercury (Hg) water	USEPA 1631	CVAF		0.2 (ng/l)	
Methyl Mercury (Hg) sediment, water	USEPA 1630	CVAF	0.00002 $\mu\text{g/g}$	0.03 (ng/l)	

Acid volatile sulfides analysis used a colorimetric method in which the sulfide in the sample was converted to hydrogen sulfide by the addition of hydrochloric acid at room temperature. The hydrogen sulfide (H₂S) was purged from the sample by an inert gas and trapped in a sodium hydroxide (NaOH) solution. With the addition of a mixed-diamine reagent (MDR), the sulfide was converted to methylene blue and measured on a spectrometer. The acid-sediment slurry was decanted into a centrifuge tube and centrifuged to settle the sediment. The supernatant was poured

into an acid cleaned Teflon bottle, ready to be analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), silver (Ag) or zinc (Zn) following a modification of EPA Method 1638; and by Cold Vapor Atomic Fluorescence (CVAF) for Hg following EPA Method 1631.

Ammonia was measured in the overlying water and in the pore water of the biological tests following methods referenced in Table 2-2.

2.4.2 METHYL MERCURY IN WATER AND SEDIMENT

The method used for methyl mercury (Hg) followed Bloom (1989) for the determination of methyl mercury in a wide range of biological and geological matrices. This CVAF technique operated in the emission of 254 nm radiation by excited Hg atoms in an inert gas stream. This method is currently contained in 1600 series for trace metals analysis (EPA Method 1630).

Sediment and pore water samples were distilled in Teflon vessels using the methods of Horvat et al. (1993). Alternatively, sediment samples can also be prepared for analysis using the method of Bloom et al. (1997). This new extraction technique avoids the methylation artifact sometimes produced in sediment sample containing high levels of inorganic mercury and organic carbon. An ethylating agent was added to the digestate or distillate to form a volatile methyl-ethyl mercury derivative and the derivative was purged onto graphitized carbon traps as a means of pre-concentration and interference removal. The mercury species were separated using isothermal chromatography, broken down to elemental mercury by means of pyrolysis, and detected using a CVAF detector as described in Bloom and Fitzgerald (1988). The detection limits were 0.00002 µg/g for sediment (0.02 ppb), and 0.03 ng/l (0.03 ppt) for water.

2.4.3 TOTAL MERCURY IN WATER

EPA Method 1631 is used routinely for the analysis of total mercury in water. This method uses a CVAF technique, based on the fluorescence of excited Hg atoms in an inert gas stream at 254 nm wavelength (Bloom and Creelius 1983). To determine total mercury, water samples were oxidized with bromine monochloride, which breaks down organo-mercury bonds. Mercuric ions in the oxidized sample were reduced to Hg with SnCl₂, and then purged onto a gold trap as a means of pre-concentration and interference removal. Mercury vapor was thermally desorbed into the fluorescence pathway. Fluorescence (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged. Typical detection limit for total mercury reported as 0.2 ng/l as Hg or 0.2 parts per trillion.

2.4.4 TOTAL MERCURY IN SEDIMENT AND TISSUE

The analysis of total mercury in sediment employs a CVAA technique based on the absorption of 254 nm radiation by excited Hg atoms in an inert gas stream. To determine total mercury, a known mass of each sample was combusted at 750°C. The evolved Hg ions were then swept into the absorption pathway. Absorption (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged. This method quantifies all mercury in the sediment including lithologic mercury. The typical detection limit for the method was 0.002 µg/g as Hg.

2.4.5 BIOACCUMULATION TISSUE CHEMISTRY

Total mercury analysis of tissues was performed to demonstrate the availability of sediment contaminants for accumulation by test organisms. Tissue composites from each replicate were analyzed separately.

2.5 BIOASSAY TESTING

Samples were evaluated in accordance with procedures outlined in the ITM (USEPA/USACE 1998) to establish suitability of sediment for disposal of dredged material in inland waters. This program included bioassay analysis of four area composite samples and two reference samples (a reference composite and one reference sample (REF X) comprised of five reference samples as independent replicates. In addition, appropriate laboratory control samples were run with each of the selected test species. Ammonia concentrations in composite sample pore-water were analyzed prior to bioassay testing in the bulk sediments. Bioassay testing for this project consists of two benthic toxicity tests, three water-column (WC) toxicity tests, and two bioaccumulation potential (BP) tests. The bioassays conducted in support of this project are summarized in Table 2-2.

Table 2-2. Biological Testing Performed for Dredged Material Evaluation.

Test Type	Type of Organism	Taxon	Project Sediments	Control Sediment/ Seawater	Reference ¹ Toxicant
Benthic	Polychaete	<i>Neanthes arenaceodentata</i>	X	X	X
	Amphipod	<i>Ampelisca abdita</i>	X	X	X
Water-Column	Fish	<i>Menidia beryllina</i>	X ²	X	X
	Mysid	<i>Americamysis bahia</i>	X ²	X	X
	Bivalve larvae	<i>Mytilus sp.</i>	X ²	X	X
Bioaccumulation Potential	Bivalve	<i>Macoma nasuta</i>	X	X	
	Polychaete	<i>Nephtys caecoides</i>	X	X	

¹Shaded areas indicate tests or treatments that are not applicable to the selected tests.

² Sediment elutriates of project material

2.5.1 BENTHIC TESTS

Benthic tests were performed to estimate the potential impact of inland water disposal of dredged material on benthic organisms that attempt to re-colonize the area. Sediment was tested using two species: the polychaete *Neanthes arenaceodentata* and the amphipod, *Ampelisca abdita*. Species of these two genera are typical inhabitants of Alaska subtidal sediments.

Juvenile polychaete worms (*N. arenaceodentata*) were supplied by Donald Reish, Ph.D., Long Beach, California. Juvenile polychaetes were held in seawater at 20°C (*Neanthes* are cultured in water-only and are not held in sediment prior to testing). Control sediment used in the benthic polychaete test was sediment from Yaquina Bay, Oregon; this sediment is native sediment supplied with the amphipod *Eohaustorius estuarius* and typically used by NewFields for control sediment in *N. arenaceodentata* testing.

Ampelisca abdita were obtained from John Brezina in Tomales Bay, California. Organisms were held at 20°C prior to testing. Native sediment was also provided and used as control sediment in the amphipod test.

Test organisms were exposed to the sediment for ten days in 1-liter glass test chambers. Two centimeters of sediment (approximately 150 mL) were placed into each chamber with 800 mL of overlying water. The bioassays were performed as static tests with no feeding during the exposure period. Initial stocking densities in each replicate were 20 organisms per test chamber for the amphipod test, and 5 organisms per test chamber for the polychaete test. Trickle-flow aeration was provided through glass pipettes, and care was taken to avoid disturbing the sediment surface. Water quality measurements were taken in one replicate for each test treatment daily and included pH, salinity, temperature, and dissolved oxygen. Ammonia was measured in both interstitial (pore water) and overlying water at the start and finish of the test from a surrogate chamber for each test treatment. Sediment pore water was extracted via centrifugation. All instruments were calibrated and logged daily. At termination, the sediments were carefully sieved to remove the test organisms and survivorship assessed using methods described in the ITM (USEPA/USACE 1998). To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980).

2.5.2 WATER-COLUMN TESTING

Water-column tests were performed to estimate the potential impact of dredged material to organisms that live in the water column. The WC test was performed using a 4:1 dilution by volume of site water to sediment. Sediment from each composite was combined with water collected from the project site, vigorously agitated for 30 minutes, and then centrifuged for approximately 30 minutes at room temperature (16–18°C). Following centrifugation, the supernatant was gently decanted. This supernatant represents the 100% test concentration and was used to create serial dilutions with clean seawater (0.45- μ m filtered Hood Canal seawater) to create subsequent test concentrations for the water-column tests. Three species were tested: *Mytilus sp.* (Bivalve larvae), *Americamysis* (formerly *Mysidopsis*) *bahia* (mysid shrimp), and *Menidia beryllina* (inland silverside fish).

The bivalve larval test was run on the test dredged material elutriates at 100%, 50%, 10%, and 1% dilutions, a clean seawater control and a site water control. There were five replicates per elutriate. The test exposure was approximately 74 hours to ensure development of the bivalve larvae to the D-hinge stage in the control. At the termination of the study, survival and normal development were compared between the control and test groups to determine if significant mortality or abnormal development occurred. The percent normal development of the test treatments were normalized for control responses.

For *A. bahia* and *M. beryllina*, the WC test was performed with dilutions of 100%, 50%, and 10% of elutriate as well as a clean seawater control and site water control under static conditions. Ten animals were used per replicate with five replicates per elutriate concentration. These tests were run for 96 hours.

Daily water quality monitoring of test chambers was carried out for pH, dissolved oxygen, salinity, and temperature. Ammonia was analyzed at the start and end of the tests in all concentrations. To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980).

2.6 ACCLIMATION OF TEST SEDIMENT

Additional testing was conducted to address acclimation of sediment to testing conditions. The acclimation efforts focused on two test composites (Area 4B based on high pore water ammonia and Lower Comp based on physical characteristics of the sediment) and the reference composite. Acclimation was required because additional contributions to toxicity may have been related to the changes in microbial processes that occur when sediment is placed into conditions established for toxicity testing that are different from conditions where the sediment was collected. Sediment such as the Lower Comp that has been deeply buried and isolated from biogenic processes (deeper than 10 cm below mud line depths) and any sediment composites that have pore water ammonia values above threshold levels eliciting a negative response in test species, need to be exposed to test conditions to allow the naturally occurring contributory factors to dissipate.

The amount of time required for acclimation is dependent on the water quality parameters of the sediment. Sediment taken from one environmental regime to another (e.g., fresh water to marine or from deep non-biogenic materials to biogenic surface material) undergoes natural microbial changes to accommodate to the new environment. A surrogate measure of the success of this process was to measure the overlying water ammonia concentration through time. The premise for using ammonia as a surrogate assumes that ammonia concentrations increase until the microbial community adjusts to the new environment. Once the microbial community was established, the overlying water ammonia concentration decreased to levels below species-specific threshold concentrations. Although, ammonia is a surrogate measure to indicate when the acclimation process was complete, acclimation of test sediment addresses other potential contributing factors including sulfide toxicity.

The differences in survival of test organisms between acclimated and unacclimated testing are attributed to the acclimation process. The premise of acclimation is that effects from the acclimated sediment represents contaminant related effects, effects from unacclimated sediment represent contributions from contaminants as well as other more transitory effects that are observed when changes occur in the biogenic nature of the sediment.

The acclimation process was performed on an additional five replicates of each test composite sample and the reference composite samples. The testing on the acclimated sediment was conducted at the same time as the standardized tests. The only difference was the period of time that the sediment was exposed to seawater before the test organisms are added to the sediment treatments. In the standard tests, sediment was exposed to seawater for one day prior to the addition of test organisms to the test containers; the acclimated sediment was exposed to seawater for approximately one week prior to the addition of test organisms.

2.6.1 BIOACCUMULATION POTENTIAL TESTING

Assessment of bioaccumulation potential was carried out using the polychaete worm *Nephtys caecoides* and the bivalve *Macoma nasuta* over a 28-day test period. Bioaccumulation tests were conducted in accordance with those procedures outlined in *Guidance Manual: Bedded Sediment Bioaccumulation Tests* (USEPA 1993) and Appendix E of the ITM (USEPA/USACE 1998). Each of these tests was initiated using test, reference, and control sediments. Five replicate tests were performed for each composite sample. *N. caecoides* exposures were conducted using 25 animals in each of five replicate test chambers. For *M. nasuta* exposures, 10 animals were placed in each of five replicate test chambers. The test chambers were maintained under flow-through conditions, and daily water quality measurements were recorded for each chamber. On Day 28, the sediment was sieved to remove the worms and clams. The surviving *M. nasuta* and *N. caecoides* were placed

in clean flow-through aquaria to purge their gut contents over 24 hours, and then tissues were placed into certified-clean glass sample jars, frozen and sent to the chemistry laboratory for tissue analysis. In order for the *N. caecoides* to purge their gut content, clean sand was also added to the clean aquaria.

The physical characteristics of the Lower Comp treatment included silty-sand sediment with very low total organic carbon content. This composite was acclimated, prior to test initiation, with raw sea water to encourage microbial growth to provide a food source for the test organism throughout the duration of the testing. The raw seawater was statically renewed daily until the start of the test and ammonia was monitored in the overlying water. One day before the start of the bioaccumulation test, the Lower Comp treatments were converted from raw seawater to filtered flowing seawater to match the set up of the other test treatments.

2.6.2 SEAWATER FOR BIOASSAY TESTING

Seawater used in this study, including the flow-through studies, came from the Hood Canal at Port Gamble, Washington. This seawater source has been used successfully on similar bioassay testing programs by the contracting team. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation from this water supply. Good survival of organisms in control sediment has been achieved consistently in previous dredge material testing conducted by the laboratory and the site is also being used to produce larval seed organisms for aquaculture purposes.

2.7 DATA MANAGEMENT AND ANALYSIS

All water quality and endpoint data were entered into Excel spreadsheets. Water quality parameters were summarized by calculating the mean, minimum, and maximum values for each test treatment. Endpoint data were calculated for each replicate and the mean value and standard deviation were determined for each test treatment.

All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the ITM (USACE/USEPA 1998) and, where appropriate, Puget Sound Dredged Material Evaluation and Disposal Procedures (USACE 2008). All statistical comparisons were performed using SAS/STAT® software (SAS Institute 2007).

All data were tested for the assumptions of normal distribution and equality of variance prior to statistical comparisons. The Shapiro-Wilk's test was used to test for normal distribution ($\alpha=0.01$, $N>20$, balanced design) and the Levene's test was performed to test for equality of variance ($\alpha=0.10$, $n=5$, balanced data).

Water column data were tested with one-tailed t-tests on arcsine-square root transformed data. Data with equal variances were compared using the combined variance; those with unequal variances used the Satterhwaite approximation for computing the test statistic. When data were not normally distributed, the t-test was performed on rankits transformed data.

Benthic survival data were tested according to both the PSSDA (USACE 2008) and ITM (USACE/USEPA 1998) methods, using arcsine-square root transformed data. PSSDA statistical

guidance calls for one-tailed t-tests on normally distributed data with either the pooled variance (equal variances) or Satterthwaite approximation (unequal variances). When data were not normally distributed, the t-test was performed on rankits transformed data. For the ITM statistics, when data met the assumptions of normality and equality of variance, an Analysis of Variance (ANOVA) with a Fisher's least significant difference (LSD) comparison on the means (one-tailed, $\alpha=0.05$) was performed. Data with normal distributions and unequal variances were tested with a one-tailed t-test (the same test as performed for PSSDA).

Concentrations of mercury in tissues exposed to test composite samples were compared to the reference composite concentrations following guidelines in the ITM (USACE 1998). All concentrations were above detection limits; therefore no censored data application was needed. When untransformed data did not meet the assumptions of normality or equal variance, the data were transformed with a natural log and retested. Data meeting both assumptions were tested with an ANOVA with a LSD comparison on the means (one-tailed, $\alpha=0.05$). When data were normally distributed but variances were unequal, individual comparisons of each test composite to the reference composite were made with a t-test using the Satterthwaite approximation for a test with unequal variance.

Comparisons of the tissue test composites were also made to the action level for mercury (0.32 ppm) requested by ADEC to address potential human health concerns and to the ERED database (USACE/USEPA 2008) maintained by the USACE – ERDC to evaluate potential ecological risk. For these comparisons, the 95% upper confidence limit on each tissue composite was calculated using the mean square error from the ANOVA when variances were equal or the variance for the sample when variances were unequal. Calculations were performed on log transformed data as appropriate and the results back-transformed for comparison to the action level.

2.8 QUALITY ASSURANCE/QUALITY CONTROL

2.8.1 FIELD SAMPLING QA/QC

Field sampling data were assessed on comparability, representativeness, and completeness. Accuracy and precision of field data were achieved by use of standardized methods of locating sampling points such as differential Global Positioning Systems, with visual verification to known landmarks. Comparability and representativeness for field sampling were achieved by use of standardized sampling equipment appropriate for the sampling location.

Field logbooks provide documentation of all sample collection activities performed. Entries were described in as much detail as possible so that persons going to the project site could reconstruct a particular sampling event. At the beginning of each field day, the date, start time, weather, names of sampling and/or investigative personnel present, were entered and signed by the person making the entry.

Information on sample collection was recorded in the logbook. All entries were made in ink. If an incorrect entry was made, the information was crossed out with a single strike mark. Wherever a sample was collected or a measurement was made, a detailed description of the location, with relevant information such that the sampling point can be relocated or mapped at a later time. Location information included GPS coordinates; any appropriate reference points and distance measurements. Any photographs taken of the station were documented. Equipment used to make field measurements were identified, along with the date of calibration.

A description of the equipment used to collect samples was entered, along with the date and time of collection, sample description, depth from which sample was collected, volume and number of containers. Sample identification numbers were assigned during sample collection. Duplicate samples received a separate sample number and were noted under the sample description.

Sample containers were provided by the analytical laboratory, who maintain documentation of the manufacturer, grade, lot number and/or other identifying information regarding preservatives added to sample containers. Chain-of-custody forms were maintained for each sample collected.

2.8.2 ANALYTICAL CHEMISTRY QA/QC

Table 2-4 lists specific data quality objectives for each group of analyses performed. The parameters used to assess data quality were precision, accuracy, representativeness, comparability, and completeness.

Table 2-3. Data Quality Objectives for Mercury Analysis

QC Measurement	Frequency	Acceptable Limits	Corrective Action
Total Mercury in Sediment and Tissue			
Method blank	1 per ≤20 samples	< 5 times the MDL	Reanalyze. If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the batch must be reanalyzed
Certified/Standard Reference Samples	1 per ≤20 samples	80-120% of certified value	Reanalyze. Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Matrix Spike	1 per ≤20 samples	80 – 120% recovery	Reanalyze. Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Replicate Precision	1 per ≤20 samples	20% for analytes > 3 times the MDL. No more than 35% of all RPDs can be >25%	Reanalyze. Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Initial and Continuing Calibration Verification	Every 10 samples	10%/20% of initial calibration	Reanalyze. If subsequent ICV or CCV still fail, rerun the calibration curve and all samples analyzed after the last passing calibration check.
Total Mercury in Aqueous Samples			
Method blank	1 per ≤20 samples	< 5 times the MDL	If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the bath must be reanalyzed
Certified/Standard Reference Samples	1 per ≤20 samples	77-123 % of certified value	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.

QC Measurement	Frequency	Acceptable Limits	Corrective Action
Matrix Spike	1 per ≤20 samples	71- 125 % recovery	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Replicate Precision	1 per ≤20 samples	21% for analytes > 3 times the MDL. No more than 35% of all RPDs can be >21%	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Initial and Continuing Calibration Verification	Every 10 samples	<15% of initial calibration	If subsequent ICV or CCV still fail, rerun the calibration curve and all samples analyzed after the last passing calibration check.
Methyl Mercury in Sediment, and Aqueous Samples			
Method blank	1 per ≤20 samples	< 5 times the MDL	If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the bath must be reanalyzed
Certified/Standard Reference Samples	1 per ≤20 samples	66-123 % of certified value	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Matrix Spike	1 per ≤20 samples	65- 135 % recovery	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Replicate Precision	1 per ≤20 samples	35% for analytes > 5 times the MDL. No more than 35% of all RSDs can be >35%	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Initial and Continuing Calibration Verification	Every 10 samples	<20% of initial calibration	If subsequent ICV or CCV still fail, rerun the calibration curve and all samples analyzed after the last passing calibration check.

The QA objective with respect to accuracy, precision, and sensitivity of laboratory data was to achieve the QC acceptance criteria of the testing protocols. In general, the accuracy and precision criteria were stipulated by the most recent versions or modifications of USEPA SW-846.

To assess the quality of data resulting from the analytical chemistry program, the following QA/QC measures were included in the sampling program:

- Procedural blanks were performed to check for artifacts associated with sample extraction and analysis. Procedural blanks will be performed at a rate of one per 20 samples or each analytical batch.
- Sufficient sample volume was supplied to the laboratory in order to perform matrix spike/matrix spike duplicate (MS/MSD). MS/MSD samples evaluated analytical accuracy and precision. MS/MSD samples were performed at a frequency of one per 20 (5%) investigative samples or each analytical batch.

- Laboratory duplicate sample analyses were performed to check precision of the analytical process. Lab duplicate samples were conducted at a frequency of one per 20 (5%) investigative samples or one per analytical batch.
- A standard reference material analysis was conducted when appropriate to evaluate the analytical accuracy. An SRM analysis was conducted at a frequency of one per 20 samples (5%) or one per analytical batch.

2.8.3 TOXICITY TESTING QA/QC

The quality assurance objectives for toxicity testing conducted by the testing laboratory are provided in detail in the ITM (USEPA/USACE 1998). These objectives for accuracy and precision involve all aspects of the testing process, included the following:

- Water and sediment sampling and handling
- Source and condition of test organisms
- Condition of equipment
- Test conditions
- Instrument calibration
- Use of reference toxicants
- Record keeping
- Data evaluation

The sensitivity of the test organisms relative to established laboratory control charts was evaluated using reference toxicant tests. The reference toxicant LC₅₀ or EC₅₀ should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits and corrective actions were taken if necessary. All limits established for this program met or are more stringent than those recommended by USEPA.

All data collected and produced were recorded on approved data sheets and became part of the permanent data record of the program. If any aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

There is no established accuracy or precision requirement for toxicity tests. Acceptable accuracy levels were generally assessed by the calibration of water quality instruments, the use of certified standards, and the establishment of acceptable water quality testing parameters. For example, water quality was monitored and, adjusted if necessary, throughout testing in at least one test replicate. Parameters that fell outside of acceptable test ranges required corrective action. Deviations from water quality testing ranges do not necessarily fail the test; however, the potential impact on test exposures was discussed.

Test organism behavior was visually monitored for each test chamber. The system was evaluated by conducting concurrent tests with negative control sediment. Adequate organism survival, as specified in the ITM (USEPA/USACE 1998), indicated a healthy testing population. Control survival for test validation was species and method specific.

To ensure that each test chamber contains the appropriate number of test organisms, a second technician checked the number of organisms in each transfer cup prior to placement in the test chamber. Duplicate counts were performed at test termination. Random allocation of test organisms and testing chambers was conducted to remove any bias associated with selectively picking the strongest organisms first or any bias associated with location of test chambers.

Representativeness was maintained during toxicity testing by ensuring that sediment was held in the dark at 4°C until needed for testing. Test sediment homogenization occurred prior to placement in test chambers. All test chambers and utensils were washed in warm soapy water, DI rinsed, acid-rinsed, and solvent rinsed. Water quality parameters were measured daily in at least one replicate per treatment. A calibration check was performed daily on all water quality instruments.

The QA objective for comparability was used to make valid comparisons with data that may be generated in the future from the project site. This objective involved the analysis of environmental samples collected during the sampling program in a manner that produced results comparable to results that would be obtained by another laboratory using the same procedures. Comparability of the data was assessed by the use of standard materials traceable to the National Institute of Standards and Technology (NIST), or approved suppliers, such as established vendors for the purchase of test organisms, the use of a positive control for toxicity tests, the use of standardized, regulatory approved procedures for sample collection and sample analysis, and analysis of quality control samples to validate the analytical results.

Each test organism batch was evaluated in reference toxicant tests during the test period to establish the sensitivity of the test organisms. The reference toxicant LC₅₀ or EC₅₀ should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

The methods employed in every phase of the toxicity testing program are detailed in the NewFields Standard Operating Practices (SOP). All NewFields staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced were recorded on approved data sheets. If an aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

The test performance criteria followed the guidance described in the ITM (USEPA/USACE1998) Section 6.1 – 6.3. The performance criteria for this project were taken *directly* from the ITM:

WATER- COLUMN TESTING PERFORMANCE CRITERIA (ITM ONLY):

- The 100% dredged material elutriate toxicity is not statistically higher than the dilution water 0%, the dredged material is not predicted to be acutely toxic to water-column organisms.
- The concentration of dissolved and suspended contaminants, after allowance for initial mixing, does not exceed 0.01 of the toxic concentration expressed as the EC or LC₅₀, beyond the boundaries of the mixing zone. Therefore the dredged material is predicted not to be acutely toxic to water column organisms. However, benthic impacts have to be considered. If information warrants, it is acceptable to determine water column effects at Tier III and benthic effects at another tier.
- The concentration of dissolved plus suspended contaminants, after allowance for mixing, exceeds 0.01 of the toxic (LC or EC₅₀) concentration beyond the boundaries of

the mixing zone. Therefore, the dredged material is predicted to be acutely toxic to water column organisms.

Water-column tests are not routinely conducted as part of the Dredged Material Evaluation and Disposal Procedures (Users Manual) (USACE 2008), therefore interpretative criteria for the water-column test will follow guidance in ITM.

BENTHIC TOXICITY TESTING PERFORMANCE CRITERIA

ITM Performance Criteria for benthic tests were predicted to be acutely toxic to benthic organisms when mean test organism mortality:

- Is statistically greater than in the reference sediment **and**
- Exceeds mortality in the reference sediment by at least 10% (...20% value for lethality can be used for amphipods, *Ampelisca abdita*, *Rhepoxynius abronius*, or *Eohaustorius estuarius* (Swartz et al, 1985; Mearns et al., 1986; SAIC, 1992 a,b).

Interpretative Criteria for the amphipod test based on the Dredged Material Evaluation and Disposal Procedures (Users Manual) (July 2008):

- Mean test mortality is significant if it is greater than 20% (absolute) over the mean negative control response, and mean test mortality is greater than 10% (dispersive) or 30% (non-dispersive) over the mean reference sediment response and statistically significant compared to reference ($\alpha = 0.5$) sediment is considered a hit

BIOACCUMULATION PERFORMANCE CRITERIA BASED ON TISSUE COMPARISONS

ITM performance guidance:

- Tissue concentrations of contaminants are not statistically less than the FDA levels. Therefore, the dredged material is predicted to result in benthic bioaccumulation of contaminants.
- Tissue concentrations of all contaminants are statistically less than FDA levels or there are no levels for the contaminants. In this case, the information is insufficient to reach a conclusion with respect to benthic bioaccumulation of contaminants. The dredged material needs to be further evaluated in Tier III as described in the subsequent bullets.
- Tissue contaminant concentrations following exposure to dredged material which are statistically less than FDA levels, or for which there are no such levels, are compared to tissue contaminant concentrations for organisms similarly exposed to reference sediment:
 - Tissue concentrations of contaminants of concern in organisms exposed to dredged material do not statistically exceed those of organisms exposed to the reference sediment; therefore, the dredged material is predicted not to result in benthic

bioaccumulation of contaminants. However, benthic toxicity effects also have to be considered.

- Tissue concentrations of contaminants of concern in organisms exposed to dredged material statistically exceed those of organisms exposed to reference material. In this case, the conclusion regarding benthic bioaccumulation of contaminants would be based upon technical evaluations that emphasize the various factors deemed appropriate in a particular region. Additional Tier IV may be required.
- Tissue concentrations are above FDA limits but are not statistically different from the reference (or disposal) site. This situation represents an exceptional case, which can only be dealt with at the regional level.

Interpretive guidance for the bioaccumulation test based on the Dredged Material Evaluation and Disposal Procedures (Users Manual) (July 2008):

- Numerical test interpretation guideline or target tissue levels (TTLs) were derived based on human health considerations. The TTLs are allowable tissue concentrations for the bioaccumulation contaminants of concern that were either derived from human-health risk assessments or from FDA action levels. The TTL for mercury is the FDA action level of 1.0 mg/kg wet weight. Interpretation of bioaccumulation results using the one-tailed one-sample t-test (alpha level = 0.05). For undetected chemicals, a concentration equal to one-half the detection limit is used.
 - If the mean tissue concentration of the contaminant of concern is greater than or equal to the TTL, then statistical testing is not required. The conclusion is that the DMMU is not acceptable for aquatic disposal.
 - If the mean tissue concentration of the contaminant of concern is less than the TTL, then a one-tailed, one-sample t-test is conducted and the DMMU is acceptable if the results are not statistically significant.

For an assessment of ecological effects, the results of the test sediment bioaccumulation responses will be compared with the bioaccumulation responses of the reference sediment. Significant bioaccumulation of chemicals of concern in test species relative to reference areas may demonstrate a potential for food-web effects.

- If the results of a statistical comparison show that the tissue concentration of the chemical of concern in test sediment is statistically higher (one-tailed, one-sample, t-test alpha level = 0.1) than the reference sediment, the DMMU will need to be evaluated further to determine the potential ecological significance of the measure tissue resides.

In addition to the performance criteria provided in both the ITM and the PSEP, ADEC requested that the bioaccumulation data be reviewed using an Alaska specific tissue concentration of total mercury of **0.32 ppm wet weight**. This value was chosen based on region-specific information

(State of Alaska Division of Public Health, 2007) and the fish consumption practices for Alaskans. The bioaccumulation data was reviewed and compared using this project specific total mercury value for tissues. The bioaccumulation data was also compared to an ecological risk related value for body burden and documented biological effects (ERED, USACE-ERDC).

3 RESULTS

3.1 FIELD SAMPLING RESULTS

Field sampling was conducted from November 17 to 21, 2008. Sediment was collected from eighteen stations within Douglas Harbor and from five different reference locations within Gastineau Channel. Table 3-1 summarizes the station location information for the Douglas Harbor samples and Figure 3-1 shows the locations on a geo-referenced map. One station, NF08-22, was estimated because coordinates were incorrectly transcribed on field logs. This station was occupied in the correct location based on visual references. The disposal site was also sampled at seven different locations to determine the overall percent fine composition of the sediment. Meg Pinza and Jay Word from NewFields and Andrew Schicht from PND conducted the field sampling. Different participants observed aspects of the field sampling including: John Stone from CBJ, Chris Meade from EPA, Brett Walters USACE and Richard Heffern from ADEC (Figure 3-2).

Table 3-1. Field Sampling Location and Collection Information

Date	Station	Composite	Latitude	Longitude	MLLW Water Depth	Number of Cores	Core Length (ft)
11/17/08	PND07-01	1	58° 16.513	134° 23.131	-6	1	10.5
11/21/08	PND07-02	1	58° 16.478	134° 23.138	+8	3	1.5/1.5/1.5
11/21/08	PND07-03	1	58° 16.494	134° 23.143	+8	3	1.5/1.5/1.5
11/18/08	PND07-04	1	58° 16.473	134° 23.182	-10	1	3.0
11/18/08	PND07-05	2	58° 16.497	134° 23.230	-9	2	4.5/3.1
11/18/08	PND07-06	2	58° 16.506	134° 23.248	-9	2	4.2/3.0
11/19/08	PND07-07	2	58° 16.489	134° 23.223	-8.5	2	2.6/1.5
11/18/08	NF08-17	2	58° 16.496	134° 23.238	-9	2	4.0/5.0
11/21/08	PND07-14	4A	58° 16.527	134° 23.185	-10	1	1.0
11/18/08	PND07-16	4A	58° 16.515	134° 23.163	-11	1	2.5
11/19/08	NF08-19	4A	58° 16.533	134° 23.221	-10.5	1	4.6
11/18/08	NF08-20	4A	58° 16.517	134° 23.189	-10.5	1	7.5
11/19/08	NF08-23	4A	58° 16.504	134° 23.151	-9	1	6.0
11/19/08	PND07-13	4B	58° 16.507	134° 23.232	-11.5	1	4.0
11/18/08	PND07-15	4B	58° 16.501	134° 23.181	-11	1	4.2
11/19/08	NF08-18	4B	58° 16.514	134° 23.237	-7.5	1	5.2
11/19/08	NF08-21	4B	58° 16.500	134° 23.207	-9	1	5.2
11/19/08	NF08-22	4B	*58° 16.485	134° 23.175	-9.5	1	4.2

* Estimated location based on visual landmarks.



Figure 3-1. Geo-referenced Locations of Sampling Stations within Douglas Harbor



Figure 3-2. Field Group Participants

The sediment samples were kept on blue ice in coolers while in transit to the laboratory in Port Gamble Washington. The sediment was received at the Port Gamble laboratory on November 28, 2008. Contents of the coolers were checked against the chain of custody form, the temperatures inside the coolers were measured upon arrival and ranged between 1 and 6°C; subsequently all samples were transferred to a cold room maintained at $4\pm 2^{\circ}\text{C}$.

3.2 SEDIMENT CORE PROCESSING

The individual cores from each field station were processed on November 30 and December 1. The sediment cores were slit vertically, the core liner spread, and the sediment was inspected. Information regarding sediment type, odor, and color were recorded on the Field Coring Logs.

During core processing distinct vertical layers of differing sediment types were noted and a decision was made to separate the upper and lower segments for each test Dredged Material Management Unit (DMMU 1, 2, 4A and 4B); Figure 3-3 shows an example of the vertical layer(s) observed. The upper layer representing up to approximately 3 feet of sediment was dark black silty organic sediment and the lower part of each core was compact grey sand with a lower percent moisture content compared to the upper sediment layer. The grey sandy sediment was also lower in total organic carbon, which posed a concern for the survival of test organisms in the longer duration bioaccumulation tests. After the sediment from each location was inspected, the sediment from each station and vertical layer was individually mixed to a homogeneous consistency and then an individual 16 oz. archive of sediment was frozen for possible future analysis. Afterwards, the sediment from each field station was combined into testing composites based on the compositing strategy described in the Douglas Harbor SAP.

Whenever two different sediment types were present in one sediment core and the upper material is softer and more pliable, it can coat the core liner from bottom to top with a slick material as the core is pushed into the sediment. This was observed in the cores from Douglas Harbor so care was taken to remove the outer sediment surface that was exposed to the core liner prior to adding sediment to the testing composites.



**Figure 3-3. A) Vertical Layers within the Sediment Core – arrow indicates location of layers
B) Removal of outer sediment surface**

The sediment from the upper sections of each station were combined into an upper test composite and the sediment from the lower section of each core were combined into a lower test composite for each of the DMMUs: 1, 2, 4A and 4B. There were eight test composites for physical and chemical analyses. Figure 3-4 shows the difference in sediment type between the upper and lower composite for Area 4B Comp.



Figure 3-4. Area 4B Upper Comp (left) and Area 4B Lower Comp (right) in Bowls

The results of the mercury analysis showed consistent and comparable concentrations in the upper and lower sediment layers. Section 3.5.2 summarizes the results of the mercury analyses. Preliminary data provided 48 hours after submittal of the sediment samples to the chemistry lab, showed that all of the mercury concentrations in the reference stations and the reference composite were below the project screening level of 0.41 mg/kg. Three test composites (Area 1 Upper Comp, Area 1 Lower Comp, and Area 2 Lower Comp) were detected below the Puget Sound Dredged Disposal Analysis Users Manual (PSDDA) maximum level of 2.1 mg/kg. The remaining test composites were above the PSDDA maximum mercury level. The sediment composition was essentially the same for each of the lower composites; grey compact sand. Therefore, it was considered an appropriate option, to allow for ample sediment for the biological testing, to combine

the material from all of the lower test composites into one testing composite (Lower Comp) for the suite of biological testing.

A variety of aquatic organisms were noted in the sediment cores including the organisms used for the bioaccumulation potential testing; the worm *Nephtys caecoides* and the clam *Macoma nasuta*. Other organisms observed in the sediment cores included a sea urchin *Strongylocentrotus drobachiensis*, several hemichordates (worm-shaped) deutrostomes or acorn worm, and mussels that were present in abundance at the sediment surface layer for many of the stations.

3.3 DISPOSAL SITE CHARACTERIZATION

On November 19, Meg Pinza, Jay Word, Andrew Schicht, Brett Walter, and Peter Wright (captain of the R/V Summer King), collected grab samples from various locations in and around the Gastineau channel. A field estimate of percent fines was determined for each location using sediment to water volume displacement method. The location of each sampling point, the water depth, and percent fines estimate are included in Table 3-2 and locations are shown on Figure 3-5.

Table 3-2. Disposal Site Sample Locations and Characteristics

Date	Station	Description	Latitude	Longitude	Depth (ft)	% Fines
11/19/2008	1	Disposal Site Corner A	58°16.7379	134°23.0205	128	65
11/19/2008	2	Outside of Disposal Site	58°16.412	134°22.408	123	82
11/19/2008	3	Within Disposal Site	58°16.706	134°22.895	128	70
11/19/2008	4	Disposal Site Corner B	58°16.6848	134°22.7908	129	80
11/19/2008	5	Disposal Site Corner C	58°16.7141	134°22.9878	125	50
11/19/2008	6	Disposal Site Corner D	58°16.6219	134°22.8145	126	79
11/19/2008	7	Middle of Disposal Site	58°16.7090	134°22.8634	126	73

Figure 3-6 and Figure 3-7 show the collection of the grab samples, the process of determining the percent fines in the field and the type of sediment present at the disposal site. Sediment consisted mainly of grey brown silt with some cobbles present. *Macoma nasuta* clam shells were present in one of the grab samples collected from the disposal site.

The sediment around the disposal site had a composition of fines that ranged from 50 to 80%. Based on this data set, the decision was made by NewFields, PND, and the regulatory agencies to consider the disposal site heterogeneous in nature. The heterogeneous nature of the disposal site determined the approach to use for collection of the reference sediment. According to the ITM (USACE 1998), if the disposal site is heterogeneous the reference approach can be used to collect reference sediment from a variety of locations and composite the material into one reference composite.

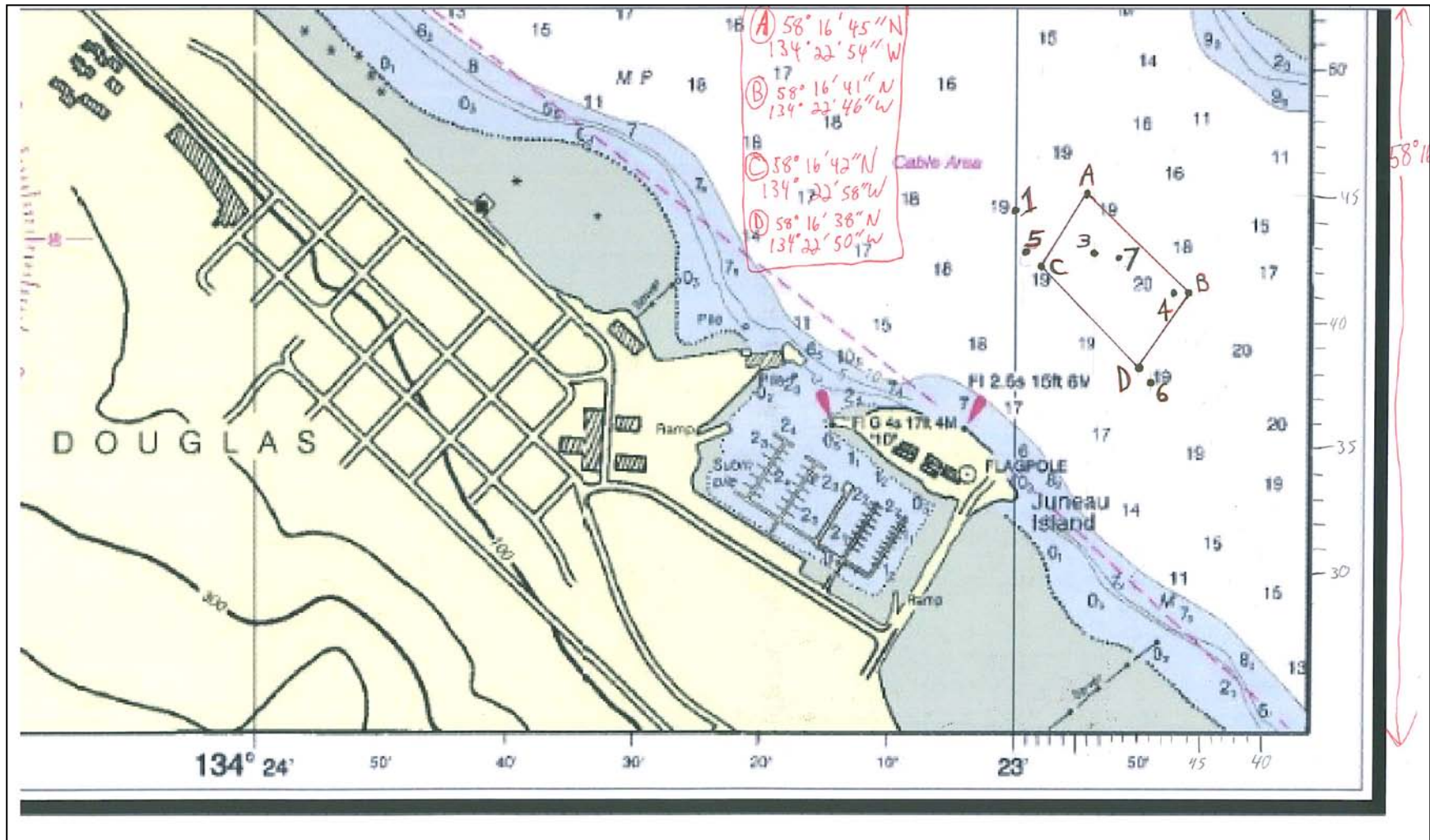


Figure 3-5. Disposal Site Sampling Locations



Figure 3-6 Brett Walters (USACE) and Jay Word (NewFields) collecting grab sample



Figure 3-7. Sediment inside of grab, sieving process, and volume displacement method

3.4 REFERENCE SEDIMENT COLLECTION

On November 20, 2008 Jay Word, Andrew Schicht, and Peter Wright (captain of R/V Summer King), collected reference sediment from five locations in Gastineau Channel. The locations of the reference sites were chosen jointly by NewFields, PND, USACE, EPA, and ADEC at a meeting held on the evening of November 19. The reference locations were chosen to be similar in nature to the disposal site with respect to sediment composition, water depth, total organic carbon, and expected infaunal community. These selected reference sites were also chosen based on historical metals data collected by Rudis, 1996. Locations are shown in Table 3-3 and Figure 3-8.

Table 3-3. Reference Site Locations and Characteristics

Date	Station	Latitude	Longitude	Depth (ft)	% Fines
11/20/2008	REF-01	58°13.192	134°16.224	108	62
11/20/2008	REF-02	58°13.526	134°16.548	103	67
11/20/2008	REF-03	58°13.931	134°17.344	110	55
11/20/2008	REF-04	58°14.330	134°18.055	121	70
11/20/2008	REF-05	58°14.685	134°19.002	120	80

Approximately 10 gallons of sediment were collected from each location using a van Veen grab. Sediment was collected into sediment field bags and stored on blue ice in coolers. The reference sediment was held on blue ice until arrival at the laboratory in Port Gamble Washington. The samples were processed by mixing the sediment from each reference location to a homogeneous consistency. Sediment was collected for chemical analysis from each individual reference site. After each of the five reference sites were prepared in the manner described, a reference composite was created by taking two gallons of sediment from each of the five reference sites. Allocations of sediment were taken from the reference composite and submitted for chemical analysis.

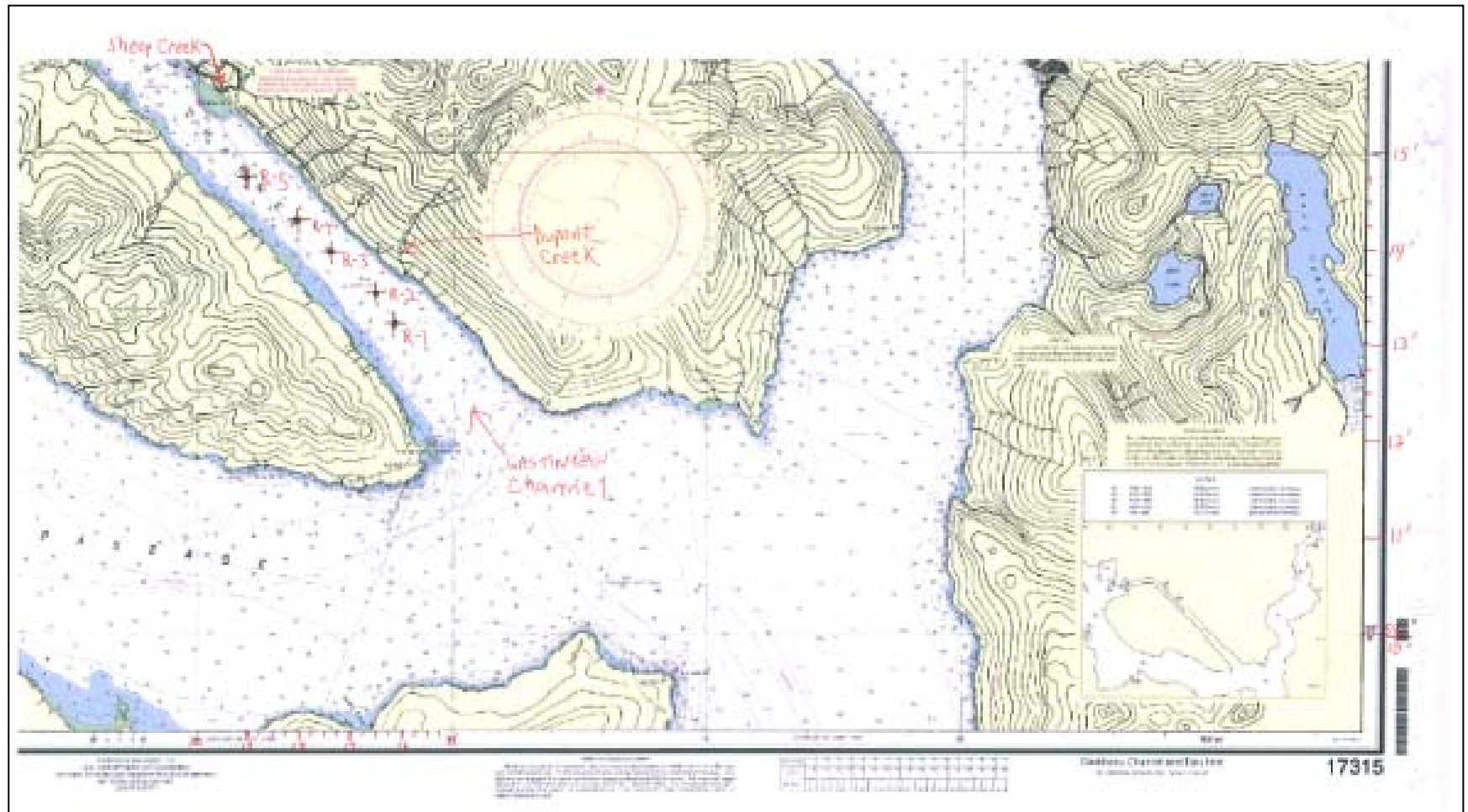


Figure 3-8. Location of Five Reference Site Samples

The consistency of the sediment from the reference areas was similar for the sites, a silty sand environment with cobble present at all locations except for REF-05. The cobble varied among sites with a range of 3 to 8 inches in diameter. The large cobbles were removed during the mixing process as they can interfere with the test results by creating pockets of anaerobic areas that could impact organism survival. A variety of organisms were noted in the sediment from the reference sites and included: brachiopods, dead coral, jingle shells, *Nephtys* sp. worms, and sipunculids. Figure 3-9 show the process of preparing the reference sediment for testing.



Figure 3-9. Sediment mixed and cobble removed. Brachiopod attached to cobble.

Because of the importance of acquiring sediment that is appropriate for the assessment type (infaunal sediment dwellers) and the lack of information on potential locations throughout Gastineau Channel, the Reference Envelope Approach was also recommended. In this case, multiple locations were sampled and handled as separate replicates for the area. This provides a comparison that would address the potential effects in an area rather than at a pre-selected point and allow separately handling the data that is obtained so that multiple sites could be examined. Ideally, all of the sediment would behave a similar manner but if there is an outlier response, that area-replicate could be removed and the data reanalyzed using an unbalanced number of replicates. Reference envelopes are being used in a variety of regions to characterize disposal site environments on a research basis (Puget Sound, San Francisco, and Columbia River). This approach combined with the Reference Area approach that produced a single composite sample from these reference locations was also performed.

3.5 PHYSICAL AND CHEMICAL ANALYSIS OF SEDIMENT

Physical-chemical analyses of the sediment included grain size, total organic carbon (TOC), total solids, and methyl and total mercury were made on subsamples collected from each composite and each station. The results of the physical and chemical characterizations are summarized in the following sections. The laboratory bench sheets for the chemical and physical analyses of sediments are presented in Appendix B.

All samples were received and analyzed within the acceptable holding times. A discussion of the QA/QC data for each analyte is presented in the following sections and in the QA/QC Summaries in Appendix B.

3.5.1 SEDIMENT GRAIN SIZE, TOTAL ORGANIC CARBON, AND TOTAL SOLIDS

Grain size results for each of the test and reference composites and individual reference stations are presented in Table 3-4. The Douglas Harbor test sediment cores were split into two test composites based on apparent grain-size with the finer silt and clay material in the upper composites and the silt and sand material in the lower composites. The grain size analysis confirmed the difference in grain size between the upper and lower layers. The upper composites with the exception of Area 1 Upper Comp were greater than 86% silt and clay while the lower composites samples were greater than 90% sand and silt. The location for Area 1 was located on the north side of the harbor and was exposed at low tide when samples were collected. The higher sand content is not unexpected given its location.

The percentage of silt and clay from the reference sites ranged from 54.7% at REF-02 to 94.4% at REF-05 encompassing the range of grain sizes observed for the Douglas Harbor test composites.

The total organic carbon content was also different between the top and bottom layers of each composite with the TOC levels in the bottom composites ranging from 0.047 to 0.069 and 0.621 to 1.88 in the upper composites. The highest TOC was associated with Area 1 Upper Comp which again may be related to the fact that it was an exposed site at low tide, with marine plant growth present on the sediment surface.

Total organic carbon in the individual reference stations and the reference composite were similar to the upper test composites with values ranging from 0.544 to 0.919. Percent solids were calculated for all of the test and reference composites with a pattern of more water in the upper composite sediment (61.0 to 64.3% solids) than in the lower composite sediments (80.8 to 84% solids). Again the individual reference samples and reference composite most closely aligned with the upper composites with total solids ranging from 50.4 to 64.9%.

ARI conducted the physical analysis of the sediment and included a matrix spike, a duplicate, a laboratory control sample, a method blank, and a standard reference material with the batch of TOC samples. The individual sample from REF-03 was used for the matrix spike and the duplicate analysis and all of the quality assurance data are provided in Appendix B. The matrix spike percent recovery was 121.3%, the relative percent difference for total organic carbon and total solids were 3.5% and 2.6% respectively, the laboratory control sample percent recovery was 101.6%, the blanks had undetected concentrations of total organic carbon, and the percent recovery of the total organic carbon standard reference material (SRM) was 92.5%. All of these measures are within the quality criteria established by the method indicating the data are usable for interpretation.

Table 3-4. Summary of Conventional Information, Douglas Harbor

Sample ID	% Gravel	% Sand	Silt (%)	Clay (%)	TOC (%)	% Solids
Area 1 Upper Comp	14.5	46.0	27.4	12.0	1.88	64.3
Area 1 Lower Comp	0.4	41.6	50.8	7.3	0.067	84.0
Area 2 Upper Comp	0.7	14.6	63.4	21.3	0.621	65.8
Area 2 Lower Comp	0.0	47.4	49.5	2.9	0.047	81.0
Area 4A Upper Comp	2.9	11.6	60.5	25.2	0.798	61.1
Area 4A Lower Comp	0.2	34.3	56.2	9.2	0.069	81.8
Area 4B Upper Comp	2.4	10.9	65.1	21.7	0.837	63.8
Area 4B Lower Comp	0.0	23.3	68.9	7.8	0.055	80.3
REF -01	14.0	26.5	38.5	20.9	0.562	63.0
REF-02	19.3	26.0	34.9	19.8	0.544	64.9
REF-03	6.1	28.3	42.3	23.4	0.687	60.1
REF-03 Lab Dup	NA	NA	NA	NA	0.646	62.7
REF-03 Lab Dup	NA	NA	NA	NA	0.647	63.1
REF-04	19.4	14.8	40.3	25.4	0.735	52.9
REF-05	0.1	5.6	61.0	33.4	0.919	50.4
REF-05 Lab Dup	0.3	5.8	60.5	33.4	NA	NA
REF-05 Lab Dup	0.0	6.2	60.2	33.6	NA	NA
REF-Comp	8.3	20.5	45.7	25.4	0.706	60.0

3.5.2 METHYL MERCURY AND TOTAL MERCURY IN SEDIMENT

Methyl and total mercury were analyzed in the test composites, individual reference samples and the reference composite (Table 3-5). Methyl mercury concentrations are reported in ng/g and represent the organic form of mercury that is more easily absorbed into the living tissue of aquatic organisms, is not easily eliminated, accumulates in organisms and may be transferred up the food chain. The degree to which mercury is transformed into methyl mercury and transferred up the food chain through bioaccumulation depends on factors such as water chemistry and the complexity of the food web.

The concentration of methyl mercury in the sediment ranged from 0.796 ng/g in sediment from Area 2 Lower Comp to 3.46 ng/g in sediment from Area 4A Lower Comp. The methyl mercury concentration in the individual reference samples and reference composite were lower, ranging from 0.277 in the REF Comp to 0.350 for REF-05.

Concentrations of total mercury measured in the composites were similar between the upper and lower layers and ranged from 1.11 µg/g to 3.22 µg/g. This range of total mercury concentrations were similar to those reported in the sediment samples collected in 2007 that ranged from 1.7 to 3.5 µg/g (Data taken from PND Report #062065, p. 10). The concentration of total mercury in the reference samples and reference composite were lower than those found in Douglas Harbor and ranged from 0.178 to 0.303 µg/g.

QA/QC measures were within quality control limits established in Table 2-4 for the blanks, standard reference materials, the matrix spikes and the replicate analysis, a summary of the quality assurance data is provided in Appendix B.

Table 3-5. Methyl and Total Mercury in Sediment, Douglas Harbor

Sample ID	% Dry Weight	Methyl Mercury (ng/g dry weight)	Total Mercury (µg/g dry weight)
Area 1 Upper Comp	61.8	2.47	1.11
Area 1 Lower Comp	82.9	3.05	1.29
Area 2 Upper Comp	60.1	0.802	2.50
Area 2 Lower Comp	80.7	0.796	1.97
Area 4A Upper Comp	61.6	1.34	3.22
Area 4A Lower Comp	80.8	3.46	2.21
Area 4A Lower Comp	80.8	3.33	2.56
Area 4B Upper Comp	64.9	1.08	2.33
Area 4B Lower Comp	80.9	2.44	3.18
REF -01	64.5	0.294	0.178
REF-02	63.2	0.308	0.195
REF-03	63.3	0.314	0.199
REF-04	55.9	0.445	0.268
REF-05	51.9	0.350	0.303
REF-Comp	58.7	0.277	0.226

3.5.3 AVS AND SEM METALS

Acid volatile sulfides (AVS) and simultaneously extracted metals data are used to determine whether sulfides are an important factor controlling the biological availability of metals in test sediments. The AVS in sediments bind to certain metals such that sediment-dwelling organisms are not likely to be exposed to the toxic potential of these metals. The SEM/AVS ratio is used to estimate if metals present in sediments are available for uptake into the tissues of aquatic organisms. If there is more AVS in sediments than metals, then the metals present in the sediments are not likely to cause adverse effects in the aquatic community near these sediments.

Each test composite, individual reference sample and the reference composite were analyzed for AVS and SEM metals. Data for the individual reference samples and the composites are presented in Table 3-6. For the Douglas Harbor composites, only one composite, Area 1 Lower Comp had a SEM/AVS ratio greater than one, indicating that for this composite the AVS is not sufficient to bind all of the SEM metals. For this composite the SEM metals could be available to sediment dwelling organisms. However, based on the solubility products for metals, mercury is the first to bind to AVS followed by Cu, Cd, Pb, Ni, and then Zn (Casas and Crecelius 1994). This means that the mercury is bound with the AVS and not available for aquatic organism uptake. The SEM/AVS

ratio was also greater than one for REF-01 through REF-04. However, given the very low concentrations of mercury in the reference samples, mercury was not expected to accumulate in tissues of organisms exposed to the reference site samples.

A QA/QC Summary is provided in Appendix B. QA/QC measures were all within target ranges, with a few exceptions. Trace amounts of SEM metals were detected in the blanks at concentrations below the sample concentrations. Sample concentrations that were less than three times the method detection limit are flagged with a J and should be considered estimates. One replicate pair for AVS had a calculated RPD greater than 25%. The RPD for SEM ranged from 0 to 35%. One replicate pair for cadmium had a RPD of 27% and one replicate for copper had an RPD of 35%. All other QC data, blank spikes, matrix spikes, and, SRM were within the data quality criteria set for the method.

Table 3-6. Concentrations of AVS and SEM Metals in Sediment (µmoles/g DW)

Sample ID	Dry Weight (%)	AVS (µmoles/g DW)	SEM/AVS Ratio	Cd	Cu	Hg	Ni	Pb	Zn
				SEM (µmoles/g DW)					
Area 1 Upper Comp	61.2	35.6	0.0391	0.00316	0.353	0.000167	0.137	0.131	0.765
Area 1 Lower Comp	82.2	0.319	1.12	0.000529	0.0745	0.000681	0.0393	0.0374	0.206
Area 2 Upper Comp	64.3	56.4	0.0299	0.00746	0.291	0.000401	0.156	0.133	1.10
Area 2 Lower Comp	81.0	0.701	0.479	0.000727	0.0450	0.000459	0.0372	0.0313	0.221
Area 4A Upper Comp	61.8	61.8	0.0261	0.00355	0.375	0.000194	0.147	0.162	0.923
Area 4A Upper Comp	61.8	71.7	0.0201	0.00315	0.262	0.000175	0.144	0.159	0.875
Area 4 A Lower Comp	80.9	6.50	0.0656	0.000888	0.0677	0.000591	0.0367	0.0583	0.262
Area 4B Upper Comp	61.6	69.9	0.0187	0.00298	0.243	0.0000982	0.135	0.148	0.779
Area 4B Lower Comp	80.6	4.98	0.0854	0.000680	0.0750	0.00112	0.0296	0.0882	0.231
REF -01	66.6	0.444	1.76	0.000640	0.161	0.0000643	0.111	0.0786	0.429
REF -01	64.0	0.578	1.44	0.000843	0.177	0.0000661	0.111	0.0847	0.458
REF-02	65.2	0.505	1.81	0.000731	0.191	0.0000783	0.126	0.0903	0.505
REF-03	63.4	0.506	1.69	0.000811	0.182	0.0000495	0.116	0.0924	0.462
REF-04	55.1	0.258 J	4.97	0.000826	0.447	0.000109	0.132	0.131	0.573
REF-05	52.3	6.00	0.236	0.00110	0.272	0.0000391	0.159	0.166	0.817
REF-Comp	60.2	1.62	0.611	0.000565	0.206	0.0000793	0.127	0.109	0.545

3.5.4 METHYL MERCURY AND TOTAL MERCURY IN PORE WATER

Methyl mercury and total mercury were analyzed in the pore water associated with each of the test and reference composites and the individual reference site samples (Table 3-7). Three composite samples from the lower layer were sufficiently dry that they did not produce pore water; therefore no measurements could be made for these samples. QA/QC measures were within quality control limits established in Table 2-4 for the blanks, standard reference materials, matrix spikes and the replicate analysis; a summary of the quality assurance data is provided in Appendix B.

The concentration of methyl mercury in the pore water samples from the Douglas Harbor test composites ranged from 0.225 ng/L (Area 2 Upper and Area 4B Upper) to 0.979 ng/L in 4A Lower Comp. The methyl mercury concentration in the individual reference samples and reference composite were 0.393 ng/L in the REF-Comp to 1.90 ng/L in REF-04. Total mercury concentrations ranged from 13.1 ng/L to 29.2 ng/L in Douglas Harbor composite samples and from 4.11 ng/L to 19.4 ng/L in the Reference area samples.

Table 3-7. Concentrations of Methyl and Total Mercury in Water

Sample ID	Methyl Mercury (ng/L)	Total Mercury (ng/L)
Area 1 Upper Comp	0.347	13.1
Area 1 Lower Comp	NM	NM
Area 2 Upper Comp	0.225	25.3
Area 2 Lower Comp	NM	NM
Area 4A Upper Comp	0.382	14.8
Area 4A Lower Comp	0.979	29.2
Area 4B Upper Comp	0.225	17.4
REF-01	0.405	5.10
REF-02	1.36	10.3
REF-03	0.582	10.7
REF-04	1.90	19.4
REF-05	0.147	4.11
REF-Comp	0.433	8.83
REF-Comp Dup	0.393	8.09

3.6 BENTHIC TEST RESULTS

This section presents a summary of the benthic tests conducted in support of Douglas Harbor project. All of the results and bench sheets for this test are provided in Appendix C. Ammonia and sulfide data were collected from the bulk pore water to determine if acclimation of test sediment was required; the bulk pore water measurements are summarized in Table 3-8; no pore water could be extracted from the Lower Comp. Area 4B Upper was selected for acclimation based on ammonia concentrations in the bulk pore water and the Lower Comp sample was acclimated due to the deep burial of the sediment and the potential isolation from biogenic processes. Testing of acclimated sediment in addition to the normal testing provides a measurement of the contribution of these factors to any observed toxicity.

In addition to the REF-COMP sample, the five individual reference samples were also tested; the mean of these results is referred to as REF-X in the following sections.

Table 3-8. Summary of Water Quality in the Bulk Pore Water

Sample ID	Total Ammonia (mg/L)	Total Sulfides (mg/L)	pH	Salinity (ppt)
Area 1 Upper	15.8	0.2	7.1	25
Area 2 Upper	15.9	0.486	7.7	21
Area 4A Upper	23.1	0.29	7.6	27
Area 4B Upper	36.6	0.502	7.7	27
REF-01	2.18	0.155	7.3	32
REF-02	3.4	0.267	7.3	32
REF-03	4.28	0.498	7.2	32
REF-04	4.43	0.125	7.2	32
REF-05	3.87	0.077	7.2	32
REF-Comp	2.57	0.125	7.2	32

3.6.1 RESULTS OF BENTHIC TEST WITH *AMPELISCA ABDITA*

The 10-day amphipod test with *A. abdita* was initiated on January 9, 2009 and was validated by 91% survival in the control treatment (Table 3-9). Measurements of DO, pH, salinity, and temperature were within recommended limits throughout the test (Tables 3-10 and 3-11).

The LC₅₀ for the cadmium reference-toxicant test was calculated at 0.74 mg Cd/L, this value is within the control chart limits (0.14 to 1.1 mg Cd/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory. The LC₅₀ for the ammonia reference-toxicant test was 24.7 mg/L. Ammonia values in the test treatments were all less than the LC₅₀ except for Area 4B which had an initial pore water ammonia concentration of 45.9 mg/L. According to the Puget Sound Dredged Material Evaluation and Disposal Procedures (USACE 2008), total ammonia values greater than 30 mg/L in the pore water is considered a threshold value that could require the sediment to be purged prior to testing. Instead of purging the sediment, Area 4B Comp was acclimated because of the potential high ammonia in the pore water interfering with the outcome of the test. The initial pore water ammonia value for the acclimated treatment was 6.22 mg/L. Survival for Area 4B was 87% and survival for Area 4B acclimated was 94%.

Mean survivals in the reference treatments were 93% in REF-Comp, 90% in REF-Comp acclimated, 95% in REF-X, and 96% in REF-X acclimated. Mean percentage survival in the test composites ranged from 76% to 94%. The survival data for *A. abdita* were arcsine-square root transformed prior to statistical comparison. The transformed data exhibited a normal distribution and equal distribution, therefore the statistical comparison was performed with ANOVA and LSD (see Section 2.7 for discussion of statistical methods).

Only the Lower Comp sample was significantly lower in survival ($p \leq 0.05$) than survival in the REF-Comp sediment. Survival of amphipods in the acclimated Lower Comp sediment was 94% and not statistically different than the REF-Comp survival.

Table 3-9. Survival Summary for the 10-Day Benthic Test with *Ampelisca abdita*.

Sample ID	Mean survival (%)	Standard Deviation	Significantly Less Than REF-Comp?
Control	91	4.2	--
REF-Comp	93	2.7	--
REF-Comp Acclimated	90	7.1	--
REF-X	95	6.1	--
REF-X Acclimated	96	4.2	--
Area 1 Comp	92	6.7	No
Area 2 Comp	92	5.7	No
Area 4A Comp	90	10.0	No
Area 4B Comp	87	5.7	No
Area 4B Comp Acclimated	94	6.5	No
Lower Comp	76	11.4	Yes
Lower Comp Acclimated	94	5.5	No

Table 3-10. Water Quality Summary for the 10-Day Benthic Test with *Ampelisca abdita*.

Sample ID	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	7.8	7.4	8.1	19.4	19.0	19.8	8.0	7.7	8.3	29.4	29.0	31.0
Area 1 Comp	7.8	6.8	8.0	19.4	19.0	19.8	8.1	7.9	8.3	29.5	29.0	31.0
Area 2 Comp	7.8	7.2	8.0	19.5	19.0	19.9	8.1	7.9	8.4	29.6	29.0	31.0
Area 4A Comp	7.8	7.4	8.1	19.4	19.0	19.8	8.0	7.7	8.3	29.4	29.0	31.0
Area 4B Comp	7.8	6.8	8.0	19.4	19.0	19.8	8.1	7.9	8.3	29.5	29.0	31.0
Area 4B acclimated	7.8	7.2	8.0	19.5	19.0	19.9	8.1	7.9	8.4	29.6	29.0	31.0
REF Comp	7.8	7.4	8.1	19.4	19.0	19.8	8.0	7.7	8.3	29.4	29.0	31.0
REF Comp acclimated	7.8	6.8	8.0	19.4	19.0	19.8	8.1	7.9	8.3	29.5	29.0	31.0
Lower Comp.	7.8	7.3	8.1	19.4	18.9	19.9	8.0	7.7	8.2	29.3	29.0	31.0
Lower Comp acclimated	7.7	6.5	8.0	19.4	19.0	19.0	8.0	7.8	8.1	31.0	30.0	33.0
REF-X	7.8	7.3	8.0	19.5	19.2	19.2	8.0	7.9	8.2	29.6	29.0	31.0
REF-X - acclimated	7.8	6.8	8.1	19.4	19.0	19.0	8.0	7.9	8.3	30.6	29.0	34.0

Table 3-11. Test Conditions for *Ampelisca abdita*.

Test Conditions for <i>A. abdita</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	7 week	
Acclimation of test sediment	Approximately 1 week prior test initiation for Area 4B Comp and Lower Comp	
Control sediment	Tomales Bay, California (native sediment)	
Test Species	<i>Ampelisca abdita</i>	
Supplier	John Brezina	
Date acquired	1/6/09	
Organism acclimation/holding time	4 days	
Age class	Adult	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	10-Day static	
Test dates	1/9/09 – 1/19/09	
Control water	0.45 µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 18.8 – 20.1 °C
Test Salinity	Recommended: 30 ± 2 ppt	Achieved: 29 – 31 ppt
Test dissolved oxygen	Recommended: > 4.6 mg/L	Achieved: 5.6 – 7.8 mg/L
Test pH	Recommended: 8.0 ± 0.5	Achieved: 7.8 – 8.8
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 9%
Reference Toxicant LC50	0.74 mg/L Cd	
Acceptable Range	0.14 – 1.1 mg/L	
Test Lighting	Continuous	
Test chamber	1-Liter Glass Chamber	
Replicates/treatment	5 + 2 surrogates for measuring pore water ammonia levels	
Organisms/replicate	20	
Exposure volume	175 mL sediment/ 950 mL water	
Feeding	None	
Water renewal	None	
Deviations from Test Protocol	None	

3.6.2 RESULTS OF BENTHIC TEST WITH *NEANTHES ARENACEODENTATA*

The benthic test with *N. arenaceodentata* was initiated on December 13, 2008 and was validated by 100% survival in the controls (Table 3-12). All water quality parameters fell within the acceptable limits throughout the duration of the 10-day test (Tables 3-13 and 3-14Table 3-13).

The LC₅₀ for the cadmium reference-toxicant test was calculated at 10.2 mg Cd/L, this value is within the control chart limits (2.8 – 16.2 mg Cd/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory. The LC₅₀ for the ammonia reference-toxicant test was 125.5 mg/L. The highest ammonia values measured in the test treatments was 12.1 mg/L in Area 4B Comp. The PSDDA Users Manual (2008) provides a threshold total ammonia value in the pore water of 10 mg/L at which no effects on survival or growth are expected to occur.

Mean survival was 96% in REF-Comp, and 92% for the REF-X samples. Mean percentage survival in the test composites ranged from 84% to 100%. Survival data were arcsine-square root transformed prior to statistical testing. Data were normally distributed, but variances were not equal therefore a one-tailed t-test was performed to compare to the REF-Comp sample. Results of the statistical analysis showed that none of the test composites had survival that was statistically lower than the reference composite.

Table 3-12. Survival Summary for the 10-Day Benthic Test with *Neanthes arenaceodentata*.

Sample ID	Mean survival (%)	Standard Deviation	Significantly Less Than REF-Comp?
Control	100	0.0	--
REF-Comp	96	8.9	--
REF-X	92	11.0	No
Area 1 Comp	96	8.9	No
Area 2 Comp	88	17.9	No
Area 4A Comp	92	11.0	No
Area 4B Comp	100	0.0	No
Lower Comp	84	16.7	No

Table 3-13. Water Quality Summary for the Benthic Test with *Neanthes arenaceodentata*.

Sample ID	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	6.9	6.4	7.7	19.6	19.2	19.9	8.1	7.9	8.3	30.3	29.0	32.0
REF-Comp	6.6	5.0	8.0	19.6	19.0	20.0	8.1	7.7	8.5	29.7	29.0	31.0
REF-X	6.8	6.2	7.7	19.5	19.0	19.8	8.2	7.9	8.7	29.7	29.0	31.0
Area 1 Comp	6.9	6.4	7.7	19.6	19.2	19.9	8.1	7.9	8.3	30.3	29.0	32.0
Area 2 Comp	6.6	5.0	8.0	19.6	19.0	20.0	8.1	7.7	8.5	29.7	29.0	31.0
Area 4A Comp	6.8	6.2	7.7	19.5	19.0	19.8	8.2	7.9	8.7	29.7	29.0	31.0
Area 4B Comp	6.9	6.4	7.7	19.6	19.2	19.9	8.1	7.9	8.3	30.3	29.0	32.0
Lower Comp	6.6	5.0	8.0	19.6	19.0	20.0	8.1	7.7	8.5	29.7	29.0	31.0

Table 3-14. Test Conditions for *Neanthes arenaceodentata*.

Test Conditions: <i>N. arenaceodentata</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	3 week	
Control sediment	Yaquina Bay, Oregon	
Test Species	<i>N. arenaceodentata</i>	
Supplier	Don Reish	
Date acquired	12/13/2008	
Organism acclimation/holding time	0	
Age class	Juvenile	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	10-Day static	
Test dates	12/13/2008-12/23/2008	
Control water	0.45 µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 19.0 – 20.0 °C
Test Salinity	Recommended: 30 ± 2 ppt	Achieved: 29 - 32 ppt
Test dissolved oxygen	Recommended: > 4.6 mg/L	Achieved: 5.0 – 8.0 mg/L
Test pH	Recommended: 8.0 ± 0.5	Achieved: 7.7 – 8.7
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 0%
Reference Toxicant LC50	10.2 mg/L	
Acceptable Range	2.8 – 16.2 mg/L	
Test Lighting	Continuous	
Test chamber	1-Liter Glass Chamber	
Replicates/treatment	5 + 2 surrogates for measuring porewater ammonia levels	
Organisms/replicate	5	
Exposure volume	175 mL sediment/ 950 mL water	
Feeding	None	
Water renewal	None	
Deviations	None	

3.7 WATER-COLUMN TEST RESULTS

The results of the water-column toxicity tests are presented in this section. The water-column tests were performed with the mysid shrimp, *Americamysis bahia*, the fish, *Menidia beryllina*, and the larvae of the bivalve, *Mytilus* sp.

3.7.1 RESULTS OF THE WATER-COLUMN TEST WITH *AMERICAMYSIS BAHIA*

The water-column test with *A. bahia* was initiated on December 17, 2008 and was validated by 98% survival in the seawater and site water controls (Table 3-15). All water quality parameters fell within the acceptable testing limits throughout the duration of the 96-hour test (Tables 3-16 and Table 3-17).

The LC₅₀ for the copper reference-toxicant test was calculated to be 242 µg Cu/L, this value is within the control chart limits (137 - 413 mg Cu/L) for this species, indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory.

The LC₅₀ for the ammonia reference-toxicant test was 70 mg/L. Ammonia values in the test treatments were all less than the LC₅₀, the highest concentration measured in the test treatments was 20.5 mg/L.

Mean percentage survival in the 100% concentration for each of the composites ranged from 98% to 100%, and the estimated LC₅₀ for each of the test treatments was >100%. Statistical comparison of the 100% concentrations of test treatment survival to control survival showed all five test treatments were not statistically lower in survival than the control; further, survival of *A. bahia* in all test concentrations were 98% or greater which is above the test performance criteria for control samples (90%).

Table 3-15. Survival Summary for *Americamysis bahia*.

Sample ID	Concentration (%)	Mean survival (%)	Standard Deviation	Statistically Less Than Control?
Control	0	98	4.5	--
Site Water	0	98	4.5	--
Area 1 Comp	10	96	5.5	--
	50	100	0.0	--
	100	98	4.5	No
Area 2 Comp	10	98	4.5	--
	50	98	4.5	--
	100	98	4.5	No
Area 4A Comp	10	98	4.5	--
	50	100	0.0	--
	100	100	0.0	No
Area 4B Comp	10	100	0.0	--
	50	100	0.0	--
	100	100	0.0	No
Lower Comp	10	98	4.5	--
	50	100	0.0	--
	100	100	0.0	No

Table 3-16. Water Quality Summary for the Water Column Test with *Americamysis bahia*.

Sample ID	Water-Column Conc. (%)	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	0	6.0	5.2	7.6	19.6	19.0	20.3	26.1	25.0	27.0	7.5	7.3	7.8
Site Water	0	6.2	5.3	8.7	19.6	19.2	20.0	24.9	24.0	26.0	7.7	7.4	7.9
Area 1 Comp	10	5.8	4.9	7.7	19.7	19.2	20.3	25.8	25.0	26.0	7.7	7.4	7.9
	50	5.8	4.9	7.5	19.7	19.1	20.5	25.8	25.0	26.0	7.8	7.4	7.9
	100	5.4	4.6	7.0	19.7	19.2	20.2	24.9	24.0	25.0	7.9	7.4	8.0
Area 2 Comp	10	5.7	4.9	7.5	19.8	19.2	20.7	25.8	25.0	26.0	7.7	7.5	8.0
	50	5.8	5.1	7.6	19.8	19.2	20.5	25.7	25.0	26.0	7.8	7.6	8.0
	100	5.6	4.8	7.5	19.8	19.2	21.2	24.9	24.0	25.0	7.9	7.8	8.1
Area 4A Comp	10	5.8	5.2	7.4	19.8	19.2	21.1	25.8	25.0	26.0	7.8	7.7	8.0
	50	5.8	5.2	7.3	19.8	19.2	20.7	25.8	25.0	26.0	7.8	7.7	8.0
	100	5.7	5.3	6.8	19.8	19.4	20.6	24.9	24.0	26.0	8.0	7.8	8.1
Area 4B Comp	10	5.8	5.1	7.4	19.7	19.3	20.4	25.8	25.0	26.0	7.8	7.7	8.0
	50	5.6	4.7	7.5	19.7	19.3	20.7	25.8	25.0	26.0	7.9	7.7	8.0
	100	5.5	4.7	7.4	19.7	19.2	20.5	25.7	25.0	26.0	8.0	7.8	8.1
Lower Comp	10	5.7	5.2	7.4	19.7	19.3	20.4	25.7	25.0	26.0	7.8	7.4	8.0
	50	5.8	5.0	7.7	19.7	19.3	20.3	25.8	25.0	26.0	7.8	7.5	8.0
	100	6.0	5.4	7.8	19.6	19.2	20.1	24.8	24.0	25.0	7.8	7.6	8.0

Table 3-17. Test Conditions for *Americamysis bahia*

Test Conditions: <i>A. bahia</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	4 weeks	
Test Species	<i>Americamysis bahia</i>	
Supplier	Aquatic BioSystems	
Date acquired	12/16/08	
Organism acclimation/holding	1 day	
Age class	4 days old	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	96-hour SPP	
Test dates	12/17/08 – 12/21/08	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 19.0 – 21.2°C
Test Salinity	Recommended: 25 ± 2 ppt	Achieved: 24 - 27 ppt
Test dissolved oxygen	Recommended: > 3.7 mg/L	Achieved: 4.6 – 8.7 mg/L
Test pH	Recommended: 7.8 ± 0.5	Achieved: 7.3 – 8.1
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 2%
Reference Toxicant LC50	242 µg Cu/L	
Acceptable Range	137 - 413 µg Cu/L	
Test Lighting	16- hours light, 8-hours dark	
Test chamber	600mL Glass Chamber	
Replicates/treatment	5	
Organisms/replicate	10	
Exposure volume	250mL	
Feeding	Twice daily	
Water renewal	None	
Deviations from Test Protocol	None	

3.7.2 RESULTS OF THE WATER-COLUMN TEST WITH *MENIDIA BERYLLINA*

The water-column test with *M. beryllina* was initiated on December 16, 2008 and was validated by 100% survival in the seawater control and 98% survival in the site water control (Table 3-18). All water quality parameters fell within the acceptable limits throughout the duration of the 96-hour test (Table 3-19 and Table 3-20).

The LC₅₀ for the copper reference-toxicant test was 307 µg Cu/L, and was inside the control chart limits (90 – 443 µg Cu/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory. The LC₅₀ for the ammonia reference-toxicant test was 62.1 mg/L. Ammonia values in the test treatments were all less than the LC₅₀ with highest measured ammonia concentration of 13.1 mg/L.

Mean percentage survival in the 100% concentration for each of the composite samples ranged from 96% to 100%, and the estimated LC₅₀ for each of the test treatments was >100%.

Table 3-18. Survival Summary for *Menidia beryllina*.

Sample ID	Water-Column Concentration (%)	Mean survival (%)	Standard Deviation	Statistically Less Than Control?
Control	0	100	0.0	--
Site Water	0	98	4.5	--
Area 1 Comp	10	100	0.0	--
	50	100	0.0	--
	100	100	0.0	No
Area 2 Comp	10	100	0.0	--
	50	98	4.5	--
	100	100	0.0	No
Area 4A Comp	10	98	4.5	--
	50	100	0.0	--
	100	100	0.0	No
Area 4B Comp	10	98	4.5	--
	50	96	5.5	--
	100	98	4.5	No
Lower Comp	10	100	0.0	--
	50	100	0.0	--
	100	100	0.0	No

Table 3-19. Water Quality Summary for the Water Column Test with *Menidia beryllina*.

Sample ID	Water-Column Conc. (%)	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control		6.8	6.1	7.3	19.7	18.9	20.3	26.0	25.0	27.0	7.8	7.4	7.9
Site Water		6.5	5.8	7.1	19.6	19.2	20.3	25.1	24.0	26.0	7.8	7.3	8.0
Area 1 comp	10	6.5	6.0	6.7	19.8	19.3	20.6	26.0	25.0	27.0	7.9	7.5	8.0
	50	6.6	6.1	6.9	19.7	19.2	20.8	26.0	25.0	27.0	7.9	7.6	8.1
	100	6.3	5.5	6.7	19.6	19.1	20.2	25.1	24.2	26.0	7.9	7.6	8.1
Area 2 comp	10	6.7	6.3	7.1	19.8	19.4	21.1	25.9	25.0	27.0	7.9	7.6	8.1
	50	6.5	6.1	6.8	19.8	19.5	20.8	25.7	25.0	26.0	8.0	7.7	8.1
	100	6.2	5.2	6.6	19.8	19.3	21.3	25.0	24.0	26.0	8.0	7.7	8.2
Area 4A comp	10	6.5	6.1	6.9	19.8	19.1	21.0	26.2	25.0	27.0	7.9	7.7	8.1
	50	6.5	6.2	6.7	19.7	19.4	20.8	25.9	25.0	27.0	8.0	7.7	8.1
	100	6.4	6.0	6.8	19.6	19.2	20.3	25.7	25.0	27.0	8.1	7.8	8.2
Area 4B comp	10	6.4	6.2	6.7	19.7	19.2	20.3	26.0	25.0	27.0	7.9	7.6	8.0
	50	6.2	5.8	6.6	19.7	19.3	20.5	25.7	25.0	26.0	8.1	7.7	8.2
	100	6.0	4.7	6.4	19.6	19.3	20.5	25.7	25.0	26.0	8.2	7.8	8.3
Lower Comp	10	6.3	5.8	6.9	19.6	19.2	20.5	25.7	25.0	26.0	7.9	7.7	8.1
	50	6.4	6.0	6.8	19.6	19.2	21.1	25.4	25.0	26.0	7.9	7.7	8.0
	100	6.5	6.2	6.7	19.6	19.1	20.8	25.1	24.0	26.0	7.9	7.6	8.1

Table 3-20. Test Conditions for *Menidia beryllina*.

Test Conditions: <i>M. beryllina</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	4 weeks	
Test Species	<i>M. beryllina</i>	
Supplier	Aquatic BioSystems	
Date acquired	12/13/08	
Organism acclimation/holding time	3 days	
Age class	10 days old	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	96-hour SPP	
Test dates	12/16/08 – 12/20/08	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 18.9 – 21.3°C
Test Salinity	Recommended: 25 ± 2 ppt	Achieved: 24- 27 ppt
Test dissolved oxygen	Recommended: > 3.7 mg/L	Achieved: 4.7- 7.3 mg/L
Test pH	Recommended: 7.8 ± 0.5	Achieved: 7.3 - 8.2
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 0%
Reference Toxicant LC50	307 µg Cu/L	
Acceptable Range	90- 443 µg Cu/L	
Test Lighting	16- hours light, 8-hours dark	
Test chamber	600mL Glass Chamber	
Replicates/treatment	5	
Organisms/replicate	10	
Exposure volume	250 mL	
Feeding	Once at 48 hours	
Water renewal	None	
Deviations	None	

3.7.3 RESULTS OF THE WATER-COLUMN TEST WITH *MYTILUS* SP.

The water-column test with *Mytilus sp.* was initiated on December 21, 2008 and was validated by 93.5% normal development in the control (Table 3-21). Dissolved oxygen, pH, and salinity fell within the test protocol limits throughout the duration of the 96-hour test (Tables 3-22 and 3-23). Temperature was above the recommended limits (16 ± 1 °C) for some of the test dilutions on Day 1 and Day 2. All of the dilutions that were above recommended limits were on the same shelf in the incubator. Given the high percentage of normal development in the test, reference, and control treatments, the slightly elevated temperature did not appear to have an effect on the outcome of the test.

The LC_{50} for the copper reference-toxicant test was calculated at 12.6 µg Cu/L, this value is within the control chart limits (3.6 – 18.4 µg Cu/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory.

The LC_{50} for the ammonia reference-toxicant test was 22.6 mg/L. Ammonia values in the test treatments were generally less than the LC_{50} , the highest measured concentration in the test samples was 26.5 mg/L. The ammonia values reported for Day 1 are considered suspect due to a malfunction with the ammonia probe caused by a puncture hole in the probe membrane. This puncture was found after the ammonia measurements were analyzed. The ammonia data are reported in Appendix C, and discussed in more detail in Section 4.

Mean percentage normal development in the 100% concentration for each of the test composites were 96.9% in Lower Comp, 63.2% in Area 1, 39.4% in Area 2, 16.1% in Area 4A, and 0% in Area 4B. The Site Water Control had 94.4% normal development and the Brine Control had 97.2% normal development. Mean normal development in the all other dilutions were above 93.4% for all of the samples except Area 4B where the 50% concentration produced a 60.7% normal development in the larvae.

The estimated EC_{50} for the composite treatments was >100% for Lower Comp and Area 1 and 87.3% Area 2, 74.6% Area 4A and 42.2% Area 4B. Statistical comparison to the control normal development for the 100% concentrations resulted in significant t-tests for Area 1 Comp, Area 2 Comp, Area 4A Comp, and Area 4B Comp.

Table 3-21. Normal Development Summary for *Mytilus* sp.

Sample ID	Concentration (%)	Mean Normal Development (%)	Standard Deviation	Significantly Less Than Control?
Control	0	93.5	3.5	--
Site Water	0	90.8	7.2	--
Brine Control	0	93.1	3.4	--
Area 1 Comp	1	96.0	4.1	--
	10	96.9	3.7	--
	50	96.2	4.8	--
	100	63.2	7.7	Yes
Area 2 Comp	1	99.4	0.9	--
	10	97.3	3.8	--
	50	96.5	3.5	--
	100	39.4	7.0	Yes
Area 4A Comp	1	96.8	3.3	--
	10	93.3	4.7	--
	50	96.3	4.2	--
	100	16.1	2.6	Yes
Area 4B Comp	1	98.6	3.0	--
	10	95.1	3.8	--
	50	60.7	8.4	--
	100	0.0	0.0	Yes
Lower Comp	1	98.8	1.8	--
	10	95.6	5.9	--
	50	93.4	7.2	--
	100	96.9	3.6	No

Table 3-22. Water Quality Summary for the Test with *Mytilus* sp.

Sample ID	Conc. (%)	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	0	7.8	7.0	8.7	16.3	15.1	17.1	7.8	7.5	8.0	31.0	31.0	31.0
Brine Control	0	7.4	7.2	7.6	16.5	15.2	17.2	7.8	7.6	8.0	31.0	31.0	31.0
Site Water	0	7.9	7.1	8.9	16.6	15.3	17.4	7.8	7.6	8.0	31.0	31.0	31.0
Area 1 Comp	1	7.7	7.1	8.2	16.4	15.2	17.2	7.9	7.6	8.0	30.7	30.0	31.0
	10	7.7	7.2	8.4	16.3	15.1	17.1	7.9	7.7	8.0	31.0	31.0	31.0
	50	7.6	7.2	8.0	16.4	15.2	17.2	8.0	7.9	8.1	30.7	30.0	31.0
	100	7.4	7.1	7.6	16.7	16.0	17.2	8.1	8.0	8.2	30.0	30.0	30.0
Area 2 Comp	1	7.7	7.1	8.3	16.4	15.5	17.1	8.1	7.9	8.2	30.7	30.0	31.0
	10	7.8	7.3	8.3	16.2	15.2	16.9	8.0	7.8	8.1	30.7	30.0	31.0
	50	7.5	7.1	7.9	16.4	15.5	17.0	8.1	8.0	8.2	30.7	30.0	31.0
	100	7.4	7.1	7.6	16.4	15.4	17.0	8.2	8.2	8.3	30.0	30.0	30.0
Area 4A Comp	1	7.5	6.9	8.4	16.9	15.1	17.9	7.9	7.9	8.0	30.7	30.0	31.0
	10	7.3	6.6	8.3	17.3	15.2	18.6	8.0	7.9	8.0	31.3	31.0	32.0
	50	7.2	6.7	8.0	18.3	15.3	20.4	8.1	8.0	8.2	31.0	31.0	31.0
	100	7.2	6.6	7.6	17.3	15.6	18.4	8.2	8.2	8.3	30.7	30.0	31.0
Area 4b Comp	1	7.4	7.0	8.2	17.2	15.2	18.5	8.1	7.9	8.2	30.7	30.0	31.0
	10	7.3	6.9	8.2	18.3	15.3	19.8	8.0	7.9	8.1	31.0	31.0	31.0
	50	7.2	6.9	7.9	17.0	15.1	18.3	8.2	8.1	8.2	30.7	30.0	31.0
	100	7.0	6.6	7.5	17.4	16.0	18.2	8.3	8.2	8.3	30.7	30.0	31.0
Lower Comp	1	7.3	6.7	8.2	18.2	15.5	19.8	8.1	8.1	8.1	31.0	31.0	31.0
	10	7.5	6.9	8.2	17.2	15.3	18.4	8.1	8.0	8.1	31.0	31.0	31.0
	50	7.4	7.0	7.9	17.1	15.1	18.4	8.0	8.0	8.0	31.0	31.0	31.0
	100	7.2	6.8	7.7	18.2	15.7	19.4	8.1	8.0	8.1	30.7	30.0	31.0

Table 3-23. Test Conditions for *Mytilus* sp.

Test Conditions: <i>Mytilus</i> sp.		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	5 weeks	
Test Species	<i>Mytilus</i> sp.- described as <i>M. galloprovincialis</i>	
Supplier	Carlsbad Aquafarms	
Date acquired	12/19/08	
Organism Acclimation/holding	2 days	
Age class	Larval	
Age of test animals	<4 hours	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	48-hour SPP	
Test dates	12/21/08 – 12/23/08	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 16 ± 1 °C	Achieved: 15.1 – 19.8°C
Test Salinity	Recommended: 31 ± 2 ppt	Achieved: 30 - 31 ppt
Test dissolved oxygen	Recommended: > 4.0 mg/L	Achieved: 6.6 – 8.9 mg/L
Test pH	Recommended: 8.0 ± 1	Achieved: 7.5 – 8.3
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 4.4 %
Reference Toxicant LC50	12.4 µg Cu/L	
Acceptable Range	3.4 – 18.7 µg Cu/L	
Test Lighting	16- hours light, 8-hours dark	
Test chamber	1-L Glass Chamber	
Replicates/treatment	5	
Organisms/replicate	Stocking density = 383 embryos per chamber	
Exposure volume	10mL	
Feeding	None	
Water renewal	None	
Deviations	Temperature slightly out of range for a few samples target temperature range is 16 to 18 °C highest temperature was 18.3 °C	

3.8 BIOACCUMULATION TEST RESULTS

Assessment of bioaccumulation potential (BP) was determined by a 28-day exposure to each of the treatment samples and reference samples. The BP test was conducted with the polychaete, *Nephtys caecoides* and the clam, *Macoma nasuta*. Following the laboratory exposures, the test organisms were depurated for 24-hours and then placed in certified-clean glass jars and frozen. *M. nasuta* were depurated in clean seawater in the absence of sediment, and *N. caecoides* were depurated in clean sand. Tissues were sent via courier to the chemistry laboratory for analysis.

The 28-day bioaccumulation test was initiated on January 9, 2009. Tests were validated by 100% survival in control samples for *N. caecoides* and 94% control survival for *M. nasuta* (Table 3-24). All water quality parameters fell within the target limits throughout the duration of the 28-d test (Table 3-25 and Table 3-26). Survival in the reference and test sediment samples for *M. nasuta* ranged from 96% to 100% while survival for *N. caecoides* was between 82% and 98%; indicating sufficient tissue for chemical analysis.

Table 3-24. Survival Summary for *Nephtys caecoides* and *Macoma nasuta* Tests

Sample ID	<i>N. caecoides</i>		<i>M. nasuta</i>	
	Mean Survival (%)	Standard Deviation	Mean Survival (%)	Standard Deviation
Control	100	0.0	94	5.5
REF-Comp	90	4.6	96	5.5
REF-X	94	10.4	96	8.9
Area 1 Comp	98	5.4	100	0.0
Area 2 Comp	90	8.8	98	4.5
Area 4A Comp	82	6.4	96	8.9
Area 4B Comp	90	10.4	98	4.5
Lower Comp	92	7.5	96	8.9

Table 3-25. Water Quality Summary for the 28-Day Bioaccumulation Test

Sample ID	Dissolved Oxygen (mg/L)			Temperature (°C)			Salinity (ppt)			pH		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	7.4	6.3	8.1	15.8	15.1	16.4	31.2	31	32	7.6	7.4	7.8
REF-Comp	7.5	6.8	8.3	15.8	15.1	16.2	31.2	31	32	7.7	7.5	7.8
REF-X	7.7	6.9	8.2	15.7	15.0	16.4	31.2	30	32	7.7	7.5	7.9
Area 1 Comp	7.3	6.2	8.0	15.8	15.0	16.4	31.2	31.0	32.0	7.6	7.2	7.8
Area 2 Comp	7.3	6.5	8.0	15.7	15.1	16.6	31.1	30.0	32.0	7.6	7.2	7.8
Area 4A Comp	7.3	6.3	7.9	15.7	15.1	16.4	31.2	31.0	32.0	7.7	7.3	7.8
Area 4B Comp	7.4	6.7	8.1	15.8	15.1	16.6	31.3	31.0	32.0	7.7	7.3	7.8
Lower Comp	7.4	6.7	8.1	15.8	15.1	16.6	31.3	31.0	32.0	7.7	7.3	7.8

Table 3-26. Summary of Test Conditions for 28-day Bioaccumulation Test

Test Conditions for Bioaccumulation Test		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	1 week	
Acclimation of test treatment	3 weeks under static renewal with raw seawater	
Test Species	<i>Macoma nasuta</i> and <i>Nephtys caecoides</i>	
Supplier	J & G Gunstone provided clams and John Brezina provided worms	
Date acquired	1/8/09 and 1/7/09	
Organism acclimation/holding time	2 days	
Age class	Adult	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	28-Day Bioaccumulation	
Test dates	1/9/09 – 2/6/09	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 15 ± 1 °C	Achieved: 15.0 – 16.6 °C
Test Salinity	Recommended: 32 ± 2 ppt	Achieved: 30 - 32 ppt
Test dissolved oxygen	Recommended: > 4.5 mg/L	Achieved: 6.3 – 8.3 mg/L
Test pH	Recommended: 7.8 ± 0.5	Achieved: 7.2 – 7.9
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: <i>M. nasuta</i> – 6% <i>N. caecoides</i> – 0%
Test Lighting	16- hours light, 8-hours dark	
Test chamber	10 Gallon Glass Aquarium	
Replicates/treatment	5	
Organisms/replicate	10 clams, 25 worms	
Exposure volume	5 cm of sediment, 30 L seawater	
Feeding	None	
Water renewal	Flow-Through	
Deviations	None	

3.9 TISSUE CHEMICAL ANALYSIS

Bioaccumulation tests were conducted using the test composites, the reference composite and the individual samples from the five reference locations. Based on the sediment chemistry, tissues from the bioaccumulation exposures were analyzed for mercury and lipids. Mean concentrations found the test treatments and the reference treatments are presented in Table 3-27. Mercury concentrations ranged from 0.016 ug/g (REF-Comp) to 0.213 ug/g (Lower Comp) in the tissues of *M. nasuta* and from 0.008 ug/g (REF-Comp) to 0.027 ug/g (Lower Comp) in the tissues of *N. caecoides*.

Table 3-27. Results of Mercury Analysis of Tissues

Sample ID	Rep	<i>M. nasuta</i>		<i>N. caecoides</i>	
		Lipid (%)	Mercury (µg/g wet weight)	Lipid (%)	Mercury (µg/g wet weight)
Area 1 Comp	1	0.84	0.0326	1.01	0.00947
	1 dup	NA	NA	1.02	NA
	2	0.81	0.0291	0.64	0.00782
	3	0.78	0.0268	0.57	0.00770
	4	0.54	0.0244	0.45	0.00746
	5	0.83	0.0248	1.10	0.0101
Area 2 Comp	1	0.92	0.0518	0.83	0.00961
	2	0.81	0.0534	0.72	0.0135
	3	0.70	0.0521	0.50	0.00878
	3 dup	NA	NA	0.48	NA
	4	0.81	0.0404	0.50	0.0135
	5	0.82	0.0666	1.02	0.0135
Area 4A Comp	1	0.92	0.0388	0.80	0.00941
	2	0.80	0.0459	0.44	0.00913
	3	0.76	0.0424	0.93	0.0102
	4	0.73	0.0372	1.09	0.0109
	5	0.57	0.0311	0.53	0.00902
Area 4B Comp	1	1.11	0.0329	0.66	0.00902
	2	0.73	0.0512	0.58	0.00922
	3	0.62	0.0374	0.46	0.0102
	4	0.87	0.0471	0.59	0.0102
	5	0.89	0.0404	0.55	0.00879
Lower Comp	1	0.81	0.206	0.47	0.0269
	1	NA	0.220	NA	NA
	2	1.03	0.235	0.73	0.0323
	3	0.80	0.242	0.48	0.0276
	4	0.86	0.199	0.83	0.0251
	5	0.39	0.186	0.45	0.0246
	5 dup	0.36	NA	NA	0.0248

Sample ID	Rep	<i>M. nasuta</i>		<i>N. caecoides</i>	
		Lipid (%)	Mercury ($\mu\text{g/g}$ wet weight)	Lipid (%)	Mercury ($\mu\text{g/g}$ wet weight)
REF-Comp	1	0.96	0.0186	0.72	0.00787
	2	0.83	0.0159	0.75	0.00826
	3	0.95	0.0141	0.80	0.00828
	4	0.80	0.0155	0.77	0.00752
	4 dup	NA	NA	NA	0.00750
	5	0.74	0.0148	0.47	0.00764
REF-01	1	0.93	0.0168	0.59	0.00801
REF-02	1	0.93	0.0133	0.90	0.00679
REF-03	1	0.80	0.0140	0.49	0.00879
REF-04	1	0.77	0.0159	0.75	0.00791
	dup	NA	0.0161	NA	NA
REF-05	1	0.71	0.0127	0.75	0.00799
	dup	0.70	NA	NA	NA
Control	1	0.89	0.0169	0.79	0.00666
	2	0.77	0.00950	0.83	0.00718
	3	0.68	0.00999	0.43	0.00677
	4	0.90	0.0108	0.89	0.00700
	5	0.99	0.0121	0.98	0.00730

4 DISCUSSION

The objective of this sampling and analysis program was to characterize the dredged materials from Douglas Harbor's four dredged material management units (Areas 1, 2, 4A and 4B). The primary disposal option for the dredged material from Douglas Harbor is inland aquatic disposal at the Gastineau Channel Dredged Material Disposal Site. Decision criteria for the evaluation of disposal suitability followed guidelines set forth in the Inland Testing Manual (ITM; USACE/USEPA 1998), and where appropriate, the PSDDA Users Manual (USACE 2008). The following discussion evaluates the physical, chemical, and biological data for each composite relative to the proposed inland aquatic disposal option. These documents rely on the toxicological responses of test organisms exposed to dredged material during removal and disposal through the water column and sediment that has been placed at the disposal site. Additionally, the ecological and human health considerations for project bioaccumulation of contaminants of concern are addressed using these documents and consensus guidance provided by the agencies earlier this year (USACE 2008; USACE/EPA 1998; State of Alaska Division of Public Health, 2007; and supplemental guidance provided by ADEC in their email 1/12/2009).

4.1 BENTHIC TEST SUMMARY FOR *AMPELISCA ABDITA*

The benthic test with *A. abdita* was conducted using the standard testing protocol and a suggested adjustment to that protocol to acclimate the sediment to appropriate biological conditions. As discussed during the preliminary Sampling and Analysis Planning meeting (November 5, 2008) this procedure has been successfully applied to remove contributing factors to adverse biological effects that are not associated with the persistent chemicals of concern. Some of those interfering contributing factors can be divided into three groups: persistent features, non persistent features, and non matrix characteristics. Persistent features do not easily change through time (e.g. sediment grain size, total organic carbon content, or water hardness for freshwater testing). Conversely, less persistent features produce effects that are time dependent and include such characteristics as ammonia or sulfide concentration, the quality of the organic carbon in the sediment, the pore water salinity and whether the sediment or soil is an appropriate habitat or must be acclimated prior to testing conditions. Other factors that are not physical or chemical include the selection of the appropriate test organism, the health of the test organism prior to and during testing, test organism acclimation and handling techniques. For a complete description of confounding factors and their application to sediment evaluations see Word et al. 2005.

The benthic test with *A. abdita* (Jan. 2009) was conducted using sediment both acclimated and unacclimated sediment for Area 4B, the Lower Composite sample (Lower Comp), the five individual reference stations, and the Reference -Composite (REF-Comp). Materials from Areas 1, 2, and 4A were tested only on acclimated sediment. The decision to perform both standard and acclimated sediment testing was based on providing appropriate biogenic testing conditions so that the effects of chemical contaminants could be separated from other contributing factors. The Lower Comp's characteristics that suggested this need as it was sandy with low total organic carbon content and had been buried at depths beyond the reach of benthic organisms for long periods of time. The Area 4B composite had relatively high levels of the less persistent ammonia and sulfides in their pore waters. Acclimation of these samples is expected to result in establishing a sediment that contained appropriate microorganisms that would handle test organism waste materials without permitting development of toxicologically important levels of ammonia or sulfides and would also modify some of the TOC in the sample to provide more biogenically available food for the test organisms (Spotte, 1992; Word et al, 2005). The reference area

sediments were acclimated in a similar manner as the test samples as a control measure to examine the effect of acclimation on samples that are expected to be acceptable.

The quantity of total organic carbon in the Lower Comp sediment (measured in the lower cores of each Area 1, 2, 4A, and 4B ranged from 0.047 to 0.069%) could have influenced the outcome of the test if some fraction of the total organic carbon was not available as a food source for the amphipods. They would then be stressed based on starvation. This sediment was acclimated by exposure to raw seawater in a carefully replaced static renewal system for three weeks prior to use in the amphipod test. This process allowed natural microbial populations to establish using the existing small amount of TOC to create a higher quality source of food for the amphipods.

The acclimation process for Area 4B Comp followed a different acclimation procedure to reduce any adverse influence from elevated pore water ammonia concentrations. The sediment was layered into the test chambers one week prior to testing and placed under test conditions. It has been established that sediment or soils placed into conditions that are not similar to their original source require acclimation to those conditions prior to successful testing of marine organisms (Spotte 1992). The typical ammonia pattern for sediment that is not acclimated prior to testing starts with relatively low concentrations of total ammonia in the overlying water, with increases in the ammonia concentrations after the first few days and subsequent decreases in ammonia concentrations after the microbial community is established.

The ammonia production cycle can be missed if ammonia is only measured at the start and end of a test. The lack of acclimation of sediment to test conditions has been shown to have an extensive influence on the survival of test organisms with as much as an 80% increase in toxicity when the sediments are not acclimated prior to the introduction of test organisms (Word *et al.* 2005).

The total ammonia concentrations for Area 4B Comp unacclimated and acclimated sediment are summarized in Table 4-1. The concentration of ammonia in the pore water was reduced by the acclimation process to below amphipod threshold levels of 20 mg/L.

Table 4-1. Total Ammonia Concentration Measured in Area 4B Comp, *Ampelisca abdita* Test

Treatment	Total Ammonia (mg/L) Test Day 0 Pore water	Total Ammonia (mg/L) Test Day 10 Pore water
Area 4B Comp Unacclimated	45.9	6.77
Area 4B Comp Acclimated	6.22	6.08

The acclimation of sediment composites did positively affect the survival of *A. abdita* in both composites. Survival in the Area 4B Comp increased from 87% to 94% and survival in the Lower Comp increased from 76% to 94%. The acclimation process did not change the results for the reference samples. Survival in the REF-Comp was 93% (unacclimated) and 90% (acclimated). Mean survival in the individual Reference (REF-X) samples was 95% (unacclimated) and 96% (acclimated). The water quality measurement of DO, pH, salinity, and temperature remained within target limits throughout the duration of the test.

For validated benthic toxicity tests, the ITM evaluation criteria for benthic toxicity are defined as: statistically significant increase in toxicity relative to the reference and increased mortality >20% (acceptable limit for *A. abdita*) above the reference survival. Under the PSDDA program, a test treatment will fail if mean mortality in the test is >20% more than the mean mortality in the appropriate control sediment or more than 10% above the appropriate reference and the difference is statistically significant ($p \leq 0.05$). Table 4-2 provides a summary of the amphipod test relative to the performance criteria.

Mortality in sediments from the Douglas Harbor were not statistically significantly higher in mortality than the reference sediment, and no mortalities exceeded the numerical criteria relative to the reference, therefore all test treatments pass the performance criteria in the ITM and the PSDDA methods (Table 4-2).

Table 4-2. Performance Criteria Comparison for *Ampelisca abdita*

Treatment	Mean Mortality (%)	Statistically greater than REF-Comp?	M _T -M _C	M _T -M _R	Pass ITM	Pass PSDDA
Control	9	---	---	---	---	---
REF-Comp	7	---	---	---	---	---
REF-Comp Acclimated	10	---	---	---	---	---
REF-X	5	---	---	---	---	---
REF-X Acclimated	4	---	---	---	---	---
Area 1 Comp	8	No	-1	1	Yes	Yes
Area 2 Comp	8	No	-1	1	Yes	Yes
Area 4A Comp	10	No	1	3	Yes	Yes
Area 4B Comp	13	No	4	6	Yes	Yes
Area 4B Comp Acclimated	6	No	-3	-4	Yes	Yes
Lower Comp	24	Yes	15	17	Yes	Yes
Lower Comp - Acclimated	6	No	-3	-4	Yes	Yes

4.2 BENTHIC TEST SUMMARY FOR *NEANTHES ARENACEODENTATA*

The sediment composites were not acclimated for the *N. arenaceodentata* test based on guidance provided in the DMMP clarification paper *Ammonia and Sulfide Guidance Relative to Neanthes Growth Bioassay (6/15/04)*. No effects on mortality were observed with bulk sediment ammonia values of ≤ 115 mg/Kg and total sulfides of ≤ 3.4 mg/L in the overlying water. The decision not to acclimate was confirmed by $> 84\%$ survival in all of the test treatments. The water quality measurements remained within target limits throughout the duration of the test and a summary of the ammonia and sulfide values for each composite are summarized in Table 4-3.

Table 4-3. Summary of Total Ammonia and Sulfide Concentrations for the *Neanthes arenaceodentata* Benthic Test

Treatment	Total Ammonia Overlying Water (mg/L) Day 0	Total Sulfide Overlying Water (mg/L) Day 0
Control	<0.5	0.141
REF Comp	<0.5	0.092
Area 1 Comp	0.702	0.016
Area 2 Comp	1.12	0.003
Area 4A Comp	1.82	0.009
Area 4B Comp	2.76	0.063
Lower Comp	<0.5	0.047

The ITM evaluation criteria for benthic toxicity are defined as: significant toxicity relative to the reference and mortality >10% above the reference survival. The PSDAA Users Manual (July 2008) does not provide performance criteria for the *N. arenaceodentata* 10-day test. Although the response for the lower composite survival meets the >10% portion of the criteria, the replicate data for that composite are sufficiently variable to not be statistically significant. Table 4-4 provides a summary of the polychaete test relative to the performance criteria.

Table 4-4. Performance Criteria Comparison for *N. arenaceodentata*

Treatment	Mean Mortality (%)	Statistically greater than REF-Comp?	M _T -M _C	M _T -M _R	Pass ITM
Control	0	---	---	---	---
REF-Comp	4	---	---	---	---
REF-X	8	---	---	---	---
Area 1 Comp	4	No	4	0	Yes
Area 2 Comp	12	No	12	8	Yes
Area 4A Comp	8	No	8	4	Yes
Area 4B Comp	0	No	0	-4	Yes
Lower Comp	16	No	16	12/8	Yes*

*Although the mean mortality is greater than 10% for the lower composite relative to the REF-Comp, replicate variability is sufficiently large to not be statistically significant. ITM requires that both a statistically significant increase in mortality plus an effect greater than 10% be required before the differences are biologically significant. Additionally, Comparison to REF-X mean is neither statistically significant nor >10% increase in mortality.

4.3 WATER-COLUMN SUMMARY

No significant toxicity was observed in the water column tests with *M. beryllina* or *A. bahia*, all test concentrations had greater than >96% survival and no statistically significant differences were observed in the 100% elutriate samples when compared to the control survival. In the larval development test for *Mytilus* sp., statistically significant differences were observed between the 100% elutriate concentration and the 0% elutriate (site water) for treatments Area 1, Area 2, Area 4A and Area 4B. The calculated EC₅₀ for each test composite is summarized in Table 4-5.

Table 4-5. Calculated EC50 Values for the *Mytilus* sp. Test

Calculated EC ₅₀	Area 1	Area 2	Area 4A	Area 4B	Lower Comp
	>100%	87.3	74.6	42.2	> 100 %

Table 4-6 provides the measured ammonia values in the elutriate concentrations. The highest ammonia values were observed in the 100% elutriates from Area 1 Comp and Area 4B Comp. An ammonia reference toxicant test was conducted along with the elutriate test. The measured ammonia concentrations in the reference toxicant test were higher than expected based on nominal concentrations. The calculated EC₅₀ from this reference toxicant test was 22.6 mg/L and the lowest observable effects concentration was 19.7 mg/L. These values are higher than other ammonia reference toxicant tests shown in Figure 4-1.

Table 4-6. Relative Concentrations of Ammonia Measured in the Reference Toxicant and Elutriate Test for *Mytilus* sp.

Treatment	Elutriate Conc. (%)	Measured Ammonia Concentration Day 0 (mg/L)	Measured Ammonia Concentration Day 2 (mg/L)	LOEC from Ammonia Ref Tox (mg/L)	EC ₅₀ from Ammonia Ref Tox (mg/L)
Control		2.03	<0.5	19.7	22.6
Site Water		<0.5	<0.5		
Brine Control		5.6	<0.5		
Area 1 Comp	1	<0.5	NM		
	10	10.8	<0.5		
	50	15.5	1.01		
	100	21.6	2.76		
Area 2 Comp	1	1.84	NM		
	10	4.40	<0.5		
	50	12.3	1.32		
	100	18.5	2.85		
Area 4A Comp	1	1.41	NM		
	10	3.90	<0.5		
	50	12.1	1.43		
	100	15.7	3.91		
Area 4B Comp	1	1.25	NM		
	10	5.82	<0.5		
	50	17.1	2.29		
	100	26.2	5.14		
Lower Comp	1	0.794	NM		
	10	1.06	NM		
	50	3.66	NM		
	100	5.47	<0.5		

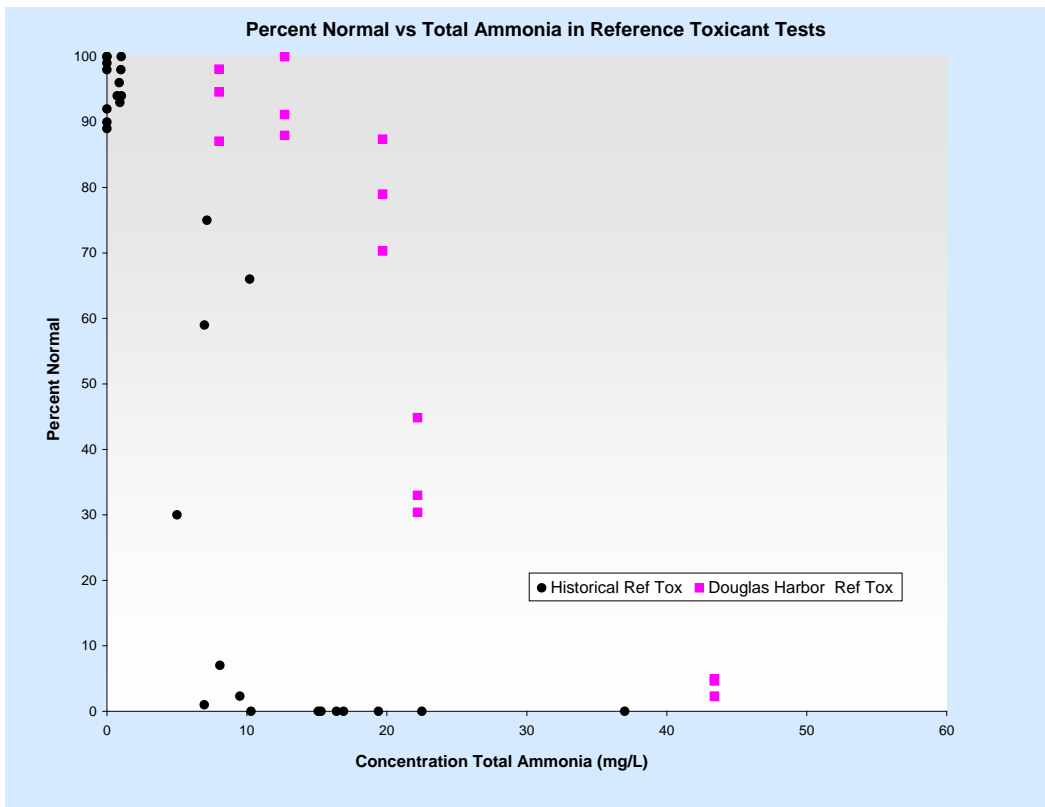


Figure 4-1. Comparison of Douglas Harbor Reference Toxicant to Historical Reference Toxicant Tests (W. Gardiner, personal communication)

The ammonia values measured on Day 0 in the elutriate test and the ammonia values for the reference toxicant test were measured on the same day using the same ammonia probe. The higher than expected ammonia readings obtained for the reference toxicant test and the higher EC₅₀ for the larval test raises concerns that the ammonia meter was not functioning properly during that time. The lab technician did find a hole in the membrane and replaced the membrane probe. The ammonia data for this test should be used only to *estimate* the relative contribution of ammonia to the test results. However, the ammonia concentrations in the 100% elutriates were above the lowest observable effects concentration of 19.7 mg/L in the reference toxicant test indicating that toxicity of these concentrations may in part be related to ammonia.

The contribution of ammonia to the overall toxicity of the elutriate preparation is not part of the decision criteria for suitability of the sediment for disposal. For the water-column tests, the performance criteria from the ITM is that the 100% elutriate concentration is not statistically higher than the 0% elutriate concentration and that the dissolved and suspended contaminants, after allowance for initial mixing, do not exceed 0.01 of the toxic concentration (expressed as the EC₅₀ or LC₅₀) beyond the boundaries of the mixing zone. The limiting permissible concentration was determined using the Short-Term Fate (STFATE) model as summarized below.

4.3.1 LIMITING PERMISSIBLE CONCENTRATION DETERMINATION

For sediment to be considered suitable for aquatic disposal the mean percentage survival or normality in the water column 100% concentrations must not be statistically significantly different than the 0% SPP treatment *and* the modeled concentration at the edge of the disposal site must not exceed Limiting Permissible Concentration (LPC). The STFATE model for dredged material disposal was used to determine whether water quality criteria would be violated during the disposal of sediments at the Gastineau Channel Disposal Site.

The LPC for the water column bioassays is one-hundredth of the acutely toxic concentration (the LC₅₀ or EC₅₀) of dredged material in the water column after the initial 1-hour mixing period. The STFATE model used for this determination is based on sediment characteristics (grain size, percent solids, and toxicity), physical oceanographic conditions at the site, the size of the designated site, and the volume of sediment to be discharged (USEPA/USACE 1998, Appendix C).

Based on the results of the larval test, the LPC for the test composites was calculated as 42.2% concentration for Area 4B Upper. This was the lowest LC₅₀ (most toxic) for any of the sites and also the finest sediment. Using the STFATE model, the LPC was calculated for the Gastineau Channel Disposal Site, a summary of the input parameters and model outputs are shown in Table 4-7. The maximum concentration at the site boundary after one hour was calculated to be 0.347%. This value is below the LPC for each of the test composites.

Table 4-7. Input Parameters to STFATE.

Calculation of Limiting Permissible Concentration Using STFATE (Ver 5.01)	
Model Input	Gastineau Channel
Mixing Area	
Depth of site (ft)	120
Width of site (Northeast to Southwest, ft)	375
Length of site (Northwest to Southeast, ft)	600
Area of site (sq ft)	225,000
Volume of disposal vessel (cu yd)	500
Length of simulation (hrs)	1
Composition of material	
Solids (%)	64.9
Sand (%)	10.9
Silt (%)	65.1
Clay (%)	21.7
Fluids (%)	35.1
Density of water (g/cc)	1.02
Water Quality Results	
Lowest LC ₅₀ or EC ₅₀ (%)	42.2
Limiting Permissible Concentration (%) = 0.01 of LC ₅₀ or EC ₅₀	0.422
Maximum concentration within mixing area during simulation (%)	0.455
Maximum concentration within mixing area at end of simulation (%)	0.0245
Maximum concentration outside disposal site during simulation (%)	0.347
Maximum concentration disposal site at end of simulation (%)	0.0245
Water Quality Criteria Violated?	No

4.4 BIOACCUMULATION SUMMARY

The 28-day bioaccumulation test was conducted using *Macoma nasuta* and *Nephtys caecoides*, two species recommended in the ITM. The ITM protocol for conducting the bioaccumulation test is 28-days. This test has been established and approved for use throughout the United States for a variety of contaminants including metals. For some organic chemicals that have a slower rate of uptake to a state of tissue equilibrium there are application factors applied to these 28-day uptake values. Mercury is not one of these; therefore the 28-day exposure period is the default time frame for ITM assessments. In the absence of a regional guidance manual, the federal manual guidance was used for this project. The rationale for the 28-day testing period is on page 6-3 through 6-5 of the ITM and summarized below:

- “The time to reach or approach steady-state varies among different compounds and, to a lesser extent among different species. Test designs that assure that steady state has been attained require a large number of samples and substantial expense. As a cost-effective compromise, it is recommended that a 28-day exposure be used for the “standard” bedded sediment bioaccumulation test for neutral organics and metals.”
- “Where it is desirable to know the steady-state concentration of neutral organic compounds as, for example, comparison to an FDA action level, fish advisory or similar numerical values, the following procedure is recommended. The log K_{ow} of the neutral organic compound of concern should be compared with the log K_{ow} in Figure 6-1 (from the ITM 1998) and will indicate the proportion of steady-state concentration (C_{ss}) expected in 28 days based on empirical evidence. This will allow estimation of the steady-state value from the 28-day laboratory exposure data using a steady-state correction factor. The correction factor is the reciprocal of the decimal fraction indicating the proportion of C_{ss} expected in 28 days.”

The octanol/water partition coefficient (K_{ow}) for methyl mercury was not provided in the ITM, therefore a list of published K_{ow} along with their citations is provided in Table 4-8.

Table 4-8. Octanol Water Partition Coefficients for Mercury

Kow	Citation
1.7	Mason et al. 1995
1.5	National Academic Press 2000

Figure 4-2 shows that Log K_{ow} values below 4.25 reach steady state within the 28-day exposure period. The low Log K_{ow} for methyl mercury suggests that a 28-day exposure is an appropriate amount of time to for any methyl mercury present in the bioaccumulation organisms to reach steady state.

Extending the bioaccumulation test beyond 28 days may have resulted in higher mortality of the test organisms due to starvation, especially for sediment with a low total organic carbon content, for example Lower Comp. This composite required acclimation (Section 2.5.1).

Discussions with CBJ, PND, and the regulatory agencies led to the acceptance the 28-day bioaccumulation protocol established by the ITM for use on Douglas Harbor sediment. Using this

established method provided a robust *scientifically defensible* data set for making decisions regarding appropriate placement of dredged material from Douglas Harbor.

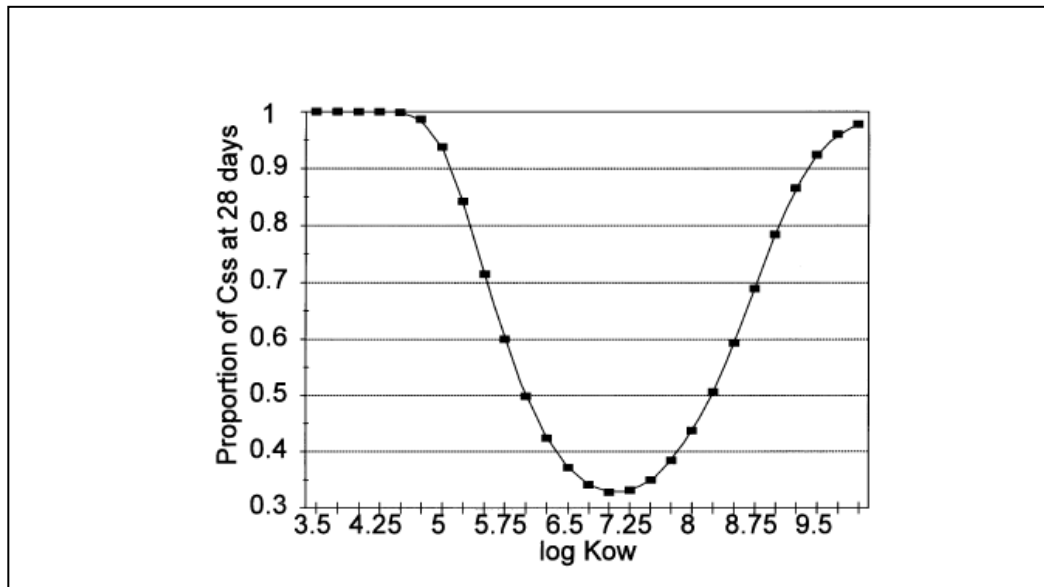


Figure 4-2. Plot of KOW and Steady State at 28-Days (USEPA/USACE 1998)

No significant toxicity was observed in the bioaccumulation tests performed on Douglas Harbor sediments. Survival in all treatments was 84% or greater, providing adequate tissue mass for chemical analyses.

Statistical analysis of test treatments compared to reference treatments showed that mercury concentrations in all test treatments were statistically significantly higher than in the REF-Comp with the exception of Area 1 Upper for *N. caecoides* (Table 4-9). The 95% upper confidence limit (UCL) for mercury in each of the composite tissue samples of *M. nasuta* and *N. caecoides* were below the 0.32 ppm wet weight threshold concentration provided by ADEC as a consensus agreement for consumption of fish and shellfish for Alaskans (Table 4-9, Figure 4-3).

In 2007 the State of Alaska Division of Public Health published the Epidemiology Bulletin Volume 11, Number 4 entitled, "*Fish Consumption Advice for Alaskans: A Risk Management Strategy to Optimize the Public's Health.*" This Bulletin includes information about mercury in fish in Alaska and gives recommended consumption allowances. The Bulletin describes an EPA screening value for unlimited consumption defined as over 16 meals per month. For 16 meals per month a monthly consumption allowance for fish of 0.32 ppm wet weight of total mercury (assumed that all mercury is methyl mercury). The consensus agreement provided by ADEC considers the 0.32 ppm as the tissue concentration number that should be used based on the Alaska fish advisory. In all cases the concentration of total mercury after the 28-day exposure period was below this consensus value for both species. In fact all of the concentrations obtained were well below the 0.15 mg/kg wet weight value except the clam for the lower composite for the unrestricted consumption value provided by the Alaska Department of Health and Social Services.

Table 4-9. Summary Statistics for Tissue Concentrations of Mercury

Composite Sample	Prob Normal ($\alpha=0.01$)	Prob Equal Variance ($\alpha=0.10$)	Prob Normal Log Transform ($\alpha=0.01$)	Prob Equal Variance Log Transform ($\alpha=0.10$)	Mean (ug/g)	Sig. Greater Than Reference ($\alpha=0.05$)	95% UCL	UCL Greater Than ADEC Action Level (0.32 ug/g)
<i>Macoma nasuta</i>								
Area 1	ANOVA / One-tailed LSD Log transformed Data	<0.001	0.652	0.882	0.027	Yes	0.031	No
Area 2					0.052	Yes	0.058	No
Area 4A					0.039	Yes	0.043	No
Area 4B					0.041	Yes	0.046	No
Lower					0.213	Yes	0.237	No
REF-Comp					0.016	--	--	
<i>Nephtys caecoides</i>								
Area 1	One-tailed T-test Log transformed Data	0.006	0.333	<0.001	0.008	No	0.010	No
Area 2					0.012	Yes	0.014	No
Area 4A					0.010	Yes	0.010	No
Area 4B					0.009	Yes	0.010	No
Lower					0.027	Yes	0.030	No
REF-Comp					0.008	--	--	

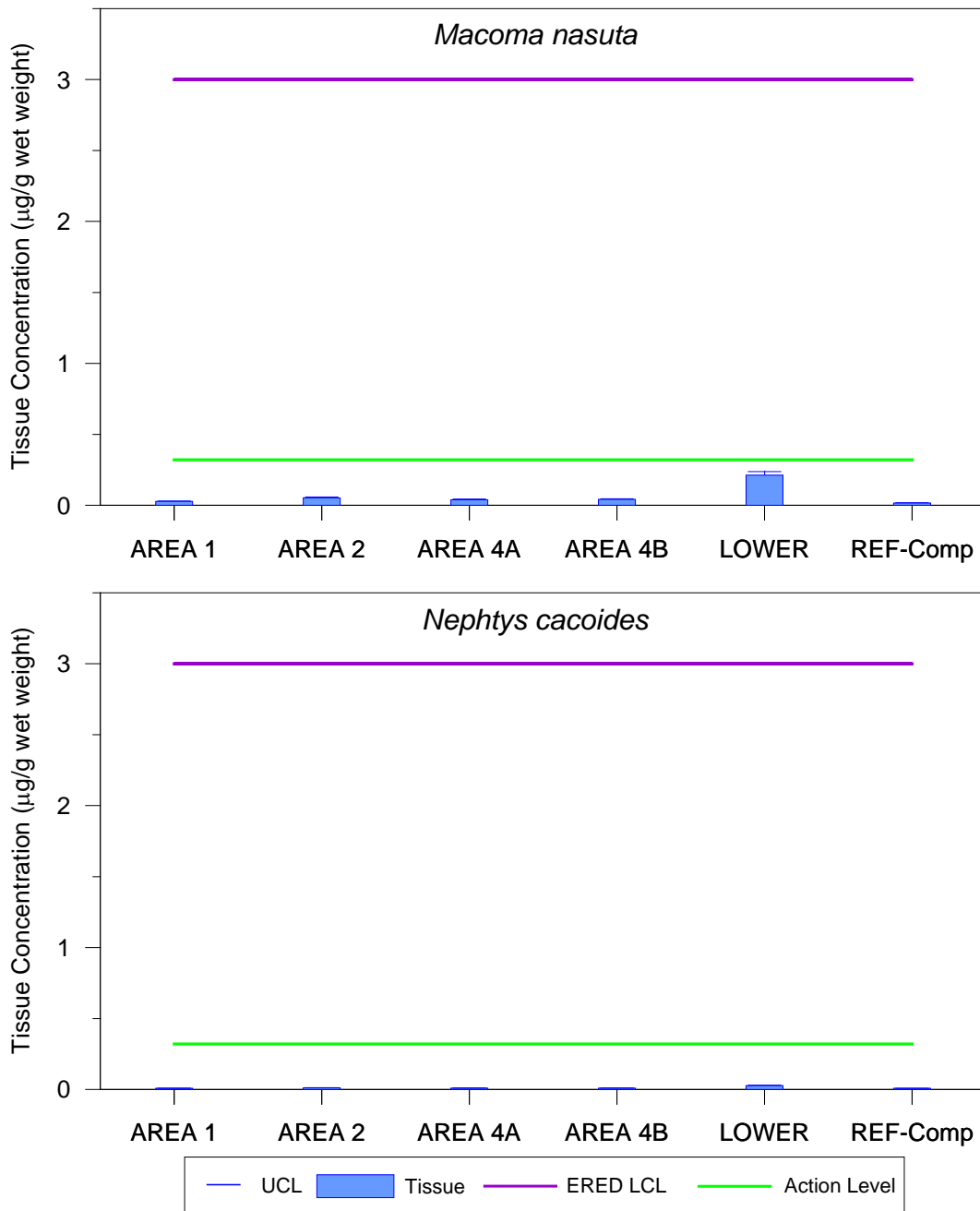


Figure 4-3. Plot of Tissue Concentrations compared to 0.32 ppm Project-Specific Action Level and ERED Lower Confidence Limit.

Bioaccumulation evaluations also examine the potential for adverse effects to organisms living at the disposal site. The tissue concentrations of the test organisms are compared to effects based values that have been developed for each chemical of concern. The Environmental Residue Effects

Data base (ERED; USACE/USEPA 2008) that is annually maintained by USACE – ERDC contains approximately 14,000 pairs of chemical specific tissue burdens to adverse biological effects that have been extracted from the scientific peer reviewed literature. In the case of mercury, the effects include development, growth, mortality and reproductive end-point evaluations. For marine organisms the most sensitive end-point is growth and the 95% LCL is ~ 3 mg/kg wet weight. The data provided by the bioaccumulation testing demonstrates that organisms that directly feed on dredged sediment from Douglas Harbor, Alaska will accumulate mercury to levels that are at least a factor of 10 below these ecological risk benchmarks.

4.5 CONCLUSIONS

The results of the Tier III/IV evaluation for Douglas Harbor included sediment chemistry, biological testing, and bioaccumulation testing. The results were compared against ITM performance criteria and PSDDSA (Users Manual) where appropriate. A summary of the findings is presented in Table 4-11 and the following paragraphs:

Mortality in the benthic amphipod and the polychaete tests was not statistically greater than in the reference and did not exceed mortality in the reference sediment by at least 10% (polychaete) or 20% for amphipod, *Ampelisca abdita* (Swartz et al, 1985; Mearns et al, 1986; SAIC, 1992 a,b); the ITM performance criteria. Using the PSDDA criteria for the amphipods, no mean test mortality was greater than 20% over the mean negative control response, and mean test mortality was not greater than 10% (dispersive) or 30% (non-dispersive) over the mean reference sediment response or statistically significant compared to reference ($\alpha = 0.5$).

The results of the 100% dredged material elutriate toxicity in the larval water column tests were statistically higher than in the dilution water. Modeling of these water column effects were demonstrated to not have effects outside of the dredged material disposal site (STFATE). The dredged material is therefore not predicted to be acutely toxic to water-column organisms and the concentration of dissolved and suspended contaminants, after allowance for initial mixing, does not exceed 0.01 of the toxic concentration expressed as the EC or LC₅₀, beyond the boundaries of the mixing zone. Therefore the dredged material is predicted not to be acutely toxic to water column organisms.

Bioaccumulation data were evaluated based on two criteria. First the concentration of bioaccumulation of a specific contaminant in dredged material exposed organisms is compared to a numerical effect limit, such as a Food and Drug Administration action level or in this case the Alaska fish advisory. If the concentration of a contaminant in a dredged material exposed organism exceeds a numerical limit, there is the potential for the dredged material disposal to have an "unacceptable adverse effect." If it does not, or there is no numerical limit, a second level of evaluation is undertaken which involves a statistical comparison of the bioaccumulation response of animals exposed to the dredged material to that of animals exposed to the reference sediment. When a statistically significant comparison is found, then a number of evaluation factors are considered to determine whether or not dredged material disposal would be predicted to result in an "unacceptable adverse effect"; including consideration of the magnitude of bioaccumulation and the toxicological significance of the bioaccumulated contaminants (USEPA/USACE 1991 and 1998).

The results of the bioaccumulation test were compared to the ITM criteria and also the PSDDA (Users Manual) criteria. The mean tissues concentration in all of the test and reference treatments were below the FDA action level of 1.0 ppm wet weight and also below the project specific target

level of 0.32 ppm wet weight. All of the test composites were statistically significantly higher than the reference composite with the exception of Area 1 Upper for *N. caecoides*.

There are limitations regarding the use of the bioaccumulation guidance, the small number of published action limits available compared to the large number of contaminants commonly present in freshwater and marine sediments and uncertainties involved in using qualitative/subjective evaluation factors. The USACE Environmental Residue-Effects Database (USACE/USEPA 2008) was developed to reduce the level of uncertainty associated with interpreting bioaccumulation data for the purpose of making regulatory decisions regarding dredged material.

The ERED database was queried for all potential ecological effects resulting from mercury exposure. The output in the form of a graph (Figure 4-4) shows that all of the published effects related to mercury are at or above 3 ppm. The most sensitive assessment end-point for mercury in marine organisms is growth and its 95% LCL is ~3 mg/kg (wet weight). The highest tissue concentration reported was 0.242 ppm (Lower Comp Rep 3) suggesting that ecological effects are not likely to be observed by organisms exposed to sediment from Douglas Harbor, Alaska when placed at the Gastineau Channel Dredged Material Disposal Site.

Table 4-10. Summary Results for Douglas Harbor Dredged Material Evaluation

Summary Results	Area 1	Area 2	Area 4A	Area 4B	Area 4B Acclimated	Lower Comp	Lower Comp Acclimated
Benthic (% survival)	<i>Douglas Harbor composites pass ITM/ PSDDA Performance Criteria</i>						
<i>A. abdita</i>	92	92	90	87	94	76	94
<i>N. arenaceodentata</i>	96	88	92	100	NA	84	NA
Water-column (LC₅₀ or EC₅₀)	<i>Water Quality Criteria Pass (STFate Model)</i>						
<i>A. bahia</i>	>100%	>100%	>100%	>100%	NA	>100%	NA
<i>M. beryllina</i>	>100%	>100%	>100%	>100%	NA	>100%	NA
<i>Mytilus. Sp.</i>	>100%	87.3	74.6	42.2	NA	> 100 %	NA
Mean Mercury Conc. (ppm)	<i>Human Health Action level is 0.32 ppm and Ecological Health is 3.0 ppm</i>						
<i>M. nasuta</i>	0.027	0.052	0.039	0.041	NA	0.213	NA
<i>N. caecoides</i>	0.008	0.012	0.010	0.009	NA	0.027	NA

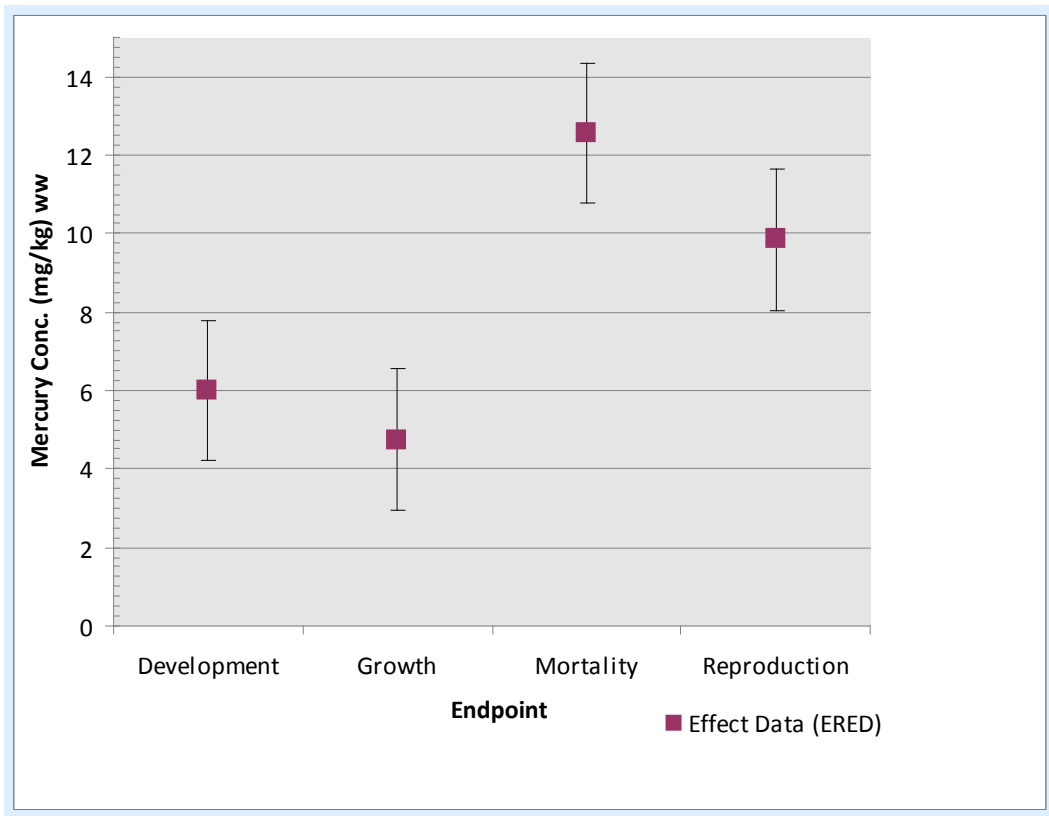


Figure 4-4. Graph from ERED Database Showing Ecological Effects Related to Mercury

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Dredged Material Evaluation for the Douglas Harbor Marina Juneau, Alaska

Final Report

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ACRONYMS

ARI	Analytical Resources Incorporated
ASTM	American Society for Testing and Materials
BP	bioaccumulation potential
CPT	cone penetration test
COC	chain of custody
DGPS	differential global positioning system
ERED	Environmental Residue-Effects Database
ERL	effects range-low
ERM	effects range-medium
ICP-MS	inductively coupled plasma emissions spectrometer equipped with a mass detector
ID	identification
ITM	Inland Testing Manual
LPC	limiting permissible concentration
MLLW	mean lower low water
POC	point of contact
QA/QC	quality assurance/quality control
QAP	quality assurance plan
SAP	sampling and analysis plan
SIM	selective ion method
SM	Standard Methods
SOP	standard operating procedure
SP	solid phase
STFATE	Short Term Fate model
TOC	total organic carbon
USACE	United States Army Corps of Engineers
USEPA	United States Environmental Protection Agency
NewFields	NewFields Northwest LLC.

UNITS OF MEASUREMENT

°C	degree(s) Celsius
ft	feet
µg/kg	microgram(s) per kilogram
µg/L	microgram(s) per liter
µm	micrometer(s)
ng/kg	nanogram(s) per kilogram
cm	centimeter(s)
L	liter
m	meter(s)
mg/kg	milligram(s) per kilogram
mL	milliliter(s)
mm	millimeter(s)
ppb	parts per billion
ppm	parts per million
ppt	parts per thousand
v/v	volume per volume
CY	cubic yards

1 INTRODUCTION

Douglas Harbor (Figure 1-1), located in Juneau Alaska, is undergoing expansion to accommodate increased moorage demands. The expansion involves removal of existing moorings, creosote pilings, and dredged material to return the harbor to its original design depth of -14 ft MLLW. The dredging aspect of the project involves the removal and disposal of approximately 30,000 cy of sediment.

PND Engineering conducted a chemical assessment of Douglas Harbor in March 2007 (Figure 1-2). Several of the samples (PND07- 13, 14, 15, and 16) were collected in the New Harbor Dredge Area and the New Surface Dredge Areas. The concentrations of mercury detected in all of the individual sediment samples and the sediment composites were above the project screening level of 0.41 mg/kg. Five of the seven composites had mercury concentrations detected above the Puget Sound Dredged Disposal Analysis Users Manual (PSDDA) maximum level of 2.1 mg/kg. The mercury concentrations were consistent throughout the entire harbor. Mercury was the only contaminant above regulatory guidance values. Biological testing was not conducted at that time.

The current project in Douglas Harbor was designed to *verify* the concentrations of mercury present in the sediment and determine if mercury concentrations in the sediment are either toxic or bioavailable to selected species of aquatic life.

The State of Alaska does not currently have a dredged material evaluation program, therefore, federal guidance provided in the Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Inland Testing Manual (ITM; USEPA/ (USACE1998) was used to conduct field sampling and laboratory testing. The results of this study should facilitate the determination of suitability of Douglas Harbor sediment for aquatic disposal at the Gastineau Channel Dredged Material Disposal Site.

The *confirmatory* chemistry and performance of biological and bioaccumulation testing of the sediment within Douglas Harbor is a Tier III evaluation with some Tier IV assessment of the bioavailability of mercury toxicity and bioaccumulation. The results of the chemical and biological analysis were evaluated according to performance criteria outlined in the ITM (USEPA/USACE 1998) and also, when applicable, the Puget Sound Dredged Material Evaluation and Disposal Procedures (Users Manual – July 2008).

1.1 BACKGROUND AND HISTORY

Douglas Harbor has undergone a number of renovations, investigations, and dredging operations since the 1940's. The last dredging program occurred in 2003, at that time dredged material was placed in the Gastineau Channel disposal site. A summary of activities related to Douglas Harbor includes:

- 1940's: Rock fill material was placed from Douglas Island to create a street out to the City wharf near the harbor entrance.
- 1948: Juneau Island Causeway was constructed along the south margin of the basin to provide vehicle access between the mining facility and Douglas Island.
- 1961: US Army Corp of Engineers (USACE) conducted site investigations for the proposed dredging of the harbor basin and for wave protection at the entrance to the harbor.

- 1962: Harbor basin was dredged to -12 ft MLLW and an entrance breakwater was constructed. Dredged material was placed on the Douglas Island side of a containment berm located along the western limits of the basin. The placement of dredge material provided a foundation for the roadways, parks, and recreational areas known today as Savikko Park.
- 1962-65: Inner harbor facilities were designed and constructed by the State of Alaska. They included Floats A, B & C, an access dock and gangway at Float B, a tidal grid and a boat ramp.
- 1995: US ACOE Civil Works conducted Tier II sampling of the harbor basin in preparation of maintenance dredging (USACE 1995).
- 1997: The US ACOE dredged approximately 25,000 cy of material in the entrance channel and northern areas of the basin. Dredged material was disposed in an unconfined manner just outside the harbor in Gastineau Channel, an inland waterway.
- 1998: The City and Borough of Juneau (CBJ) constructed seven stall floats along the north side of Float C.
- 2001-03: The CBJ expanded the Douglas Harbor basin and installed Floats D&E resulting in the current configuration. Approximately 65,000 cy of material was dredged during this effort. A majority of the dredged material (roughly 90%) was disposed behind a geotextile lined containment berm on-site creating a boat launch ramp and parking area. The remaining dredged material was disposed in an unconfined manner outside the harbor in Gastineau Channel.
- 2007-08: The CBJ is currently planning to renovate the original section of Douglas Harbor constructed during the period 1962-65. The existing harbor facilities are severely deteriorated and need to be replaced to provide safe public moorage. The current harbor basin elevation has risen, likely due to glacial rebound and dredging is necessary to maintain safe navigational depth for vessels moored in the harbor.

The 2007 PND field survey conducted sediment sampling and physical characterization combined with chemistry analyses of the following parameters and chemicals of potential ecological concern:

- Grain size
- Total volatile Solids
- Gasoline Range Organics, Diesel Range Organics, Residual Range Organics
- Benzene, Toluene, Ethylene, and Xylene
- Polynuclear Aromatic Hydrocarbons (PAH)
- Metals
- Chlorinated hydrocarbons
- Organotins

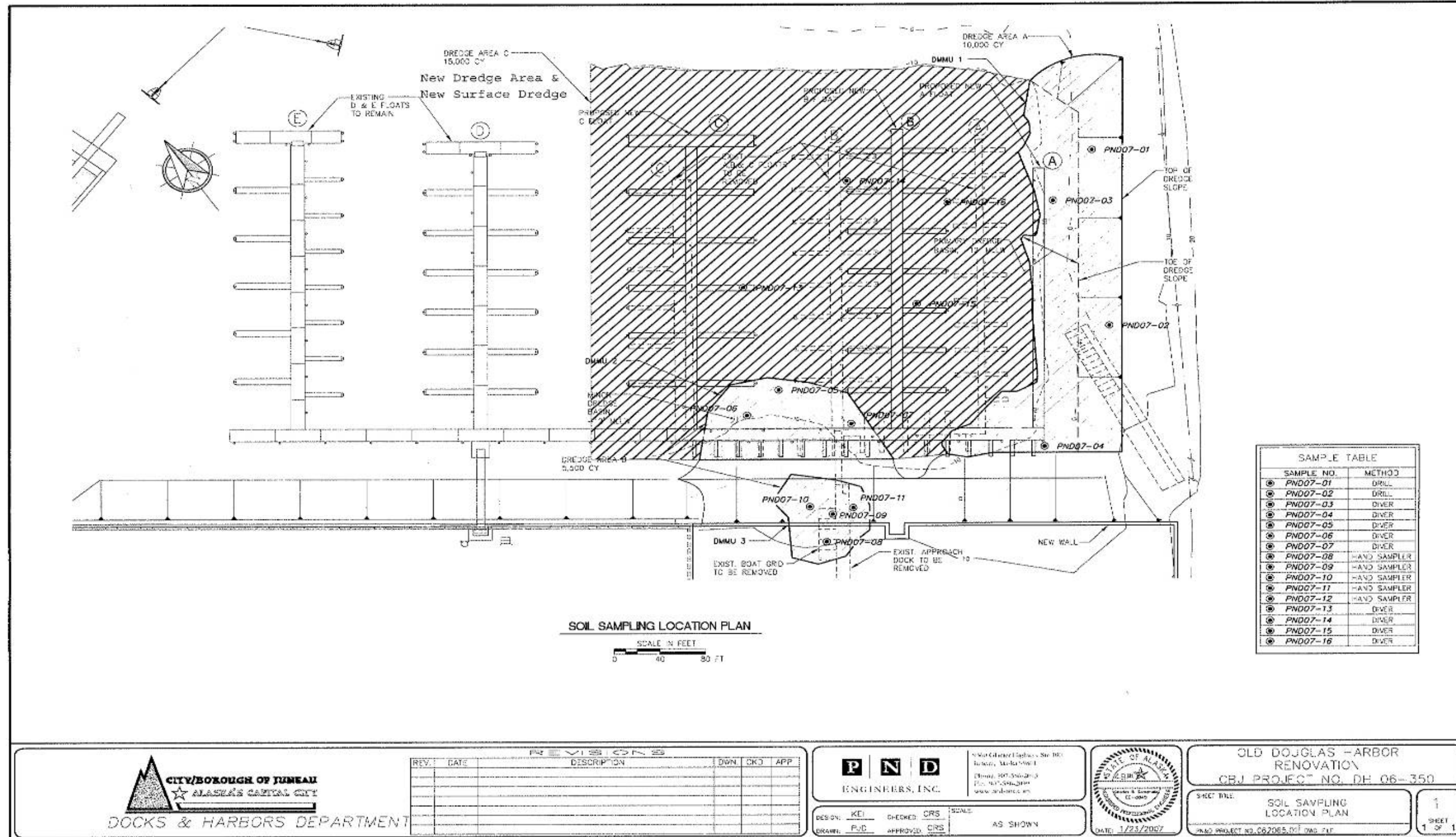


Figure 1-1. Douglas Harbor Site Map from 2007 Field Survey.

Mercury was the *only* contaminant determined to be of potential ecological concern with concentrations above the project screening level of 0.41 mg/kg and the PSDDA maximum level of 2.1 mg/kg. Mercury concentrations in the test *composites* from the 2007 survey are summarized in Table 1-1 (PND 2007; Data taken from PND Report #062065, p10). Individual sediment sample concentrations ranged from 0.47 to 5.4 mg/kg.

Table 1-1. Mercury Concentrations in Composite Sediment Samples, 2007.

Sample Location	Mercury Concentration (mg/kg dry weight)
PND-11	1.3
PND-2	2.4
PND-4	2.5
Harbor Dredge	3.5
New Surface Dredge	2.2
PND-1	1.8
PND-3	2.7

Concentrations of the other potential contaminants of concern were below screening levels and were not be analyzed as part of this program.

1.2 SAMPLING STRATEGY AND TESTING OBJECTIVES

The main objective of the current project was to verify mercury concentrations in the proposed dredged material from Douglas Harbor and to determine suitability for aquatic disposal using guidelines established in the ITM (USEPA/USACE 1998). The testing strategy paralleled the tiered testing approach (Section 3) of the ITM.

Specific project objectives were to:

- Collect test sediment to project depth using a vibratory or push core.
- Collect reference sediment from the proposed reference area (five spatial replicates and one reference composite made from five spatial replicates) using a van Veen grab.
- Conduct toxicity testing of test, reference, and control sediments using ITM methods for water-column toxicity, benthic toxicity, and bioaccumulation potential.
- Measure mercury concentrations in sediment, pore water, and tissue.
- Prepare a detailed interpretative report that includes methods, results, and a comparison of test and reference materials using ITM guidance for test acceptability and performance criteria.

Detailed sediment chemistry analysis for a variety of potential contaminants of concern was performed in 2007 as part of the Tier II assessment. The concentrations of mercury were above project screening levels; therefore this Tier III evaluation included quantification of the mercury

concentrations along with biological and bioaccumulation testing. Figure 1-2 illustrates the tiered testing approach used for this study, (figure taken directly from the ITM (USEPA/USACE 1998).

The proposed site for receipt of dredged material from Douglas Harbor is the Gastineau Channel (GC) disposal site. To determine suitability of Douglas Harbor material for disposal at this site, chemical and biological analysis included a control for test validation and reference area samples collected and tested concurrently with the test sediment following ITM procedures.

The native control sediment was specific to each type of toxicity test and species and was either collected from places where the test organisms naturally reside or was taken from cultures of test organisms in the laboratory. The response of the test organism to this sediment was used to confirm the health of the test animals and to validate the acceptability of the tests performed.

The purpose of reference sediment was to provide a point of comparison (reference point) to which benthic effects of dredged material were compared. Reference sediment was collected *outside* the influence of previous disposal operations at a dredged material disposal site, but near enough to the disposal site that the reference sediment is subject to all the same natural influences as the disposal site (USEPA/USACE 1998).

A designated reference site for the purposes of dredged material evaluation does not exist in Juneau, Alaska area. PND and the regulatory agencies (Figure 1-3) chose five different locations to represent the reference area. The five locations were tested separately and as part of a reference composite made from the five locations. There is a possibility that sediment previously disposed of at the Gastineau Channel (Figure 1-4) may have migrated outside the disposal site, therefore, the location of the reference area was placed outside of the area possibly influenced by previous disposal operations.

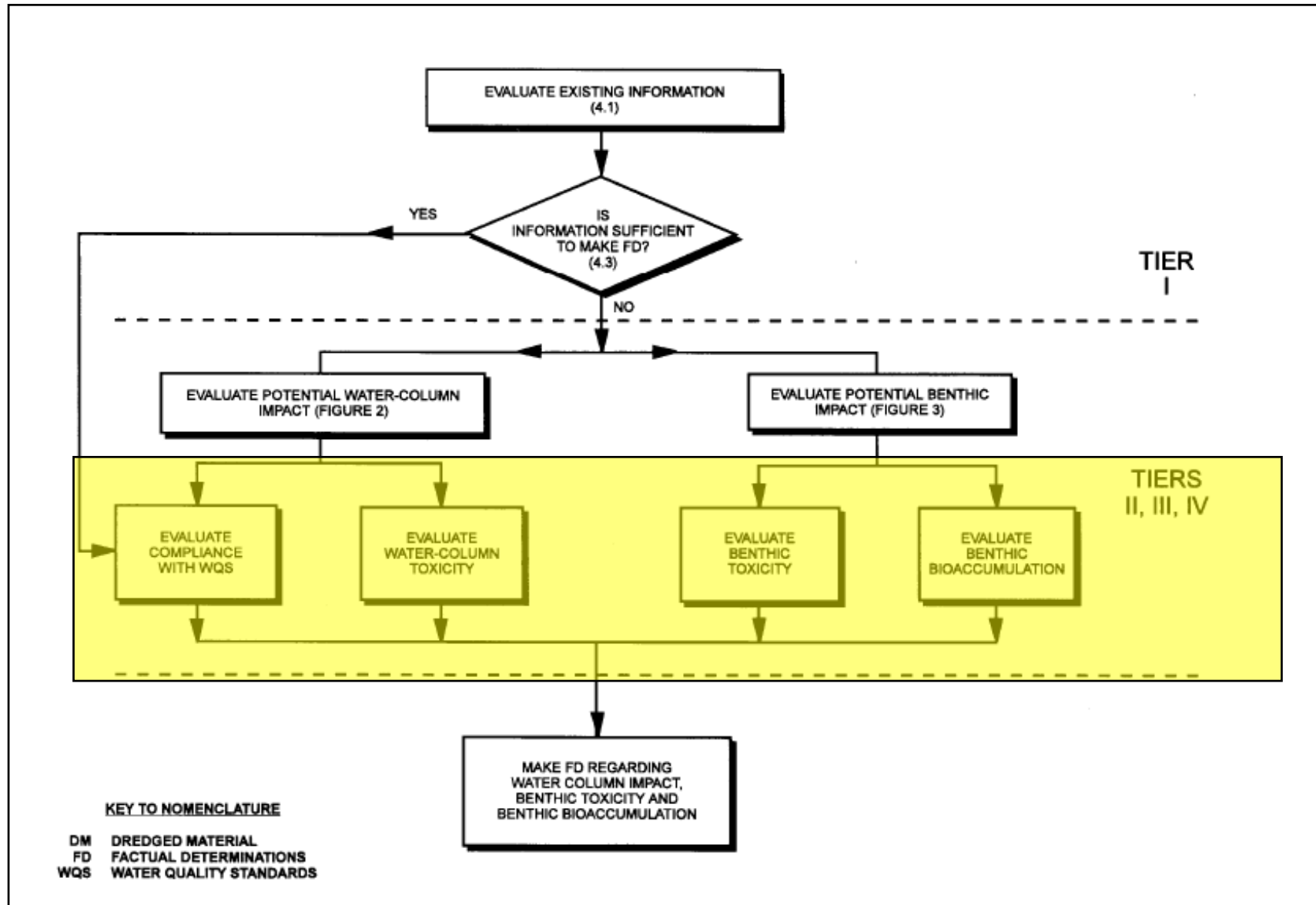


Figure 1-2. Tiered Testing Approach (ITM 1998)

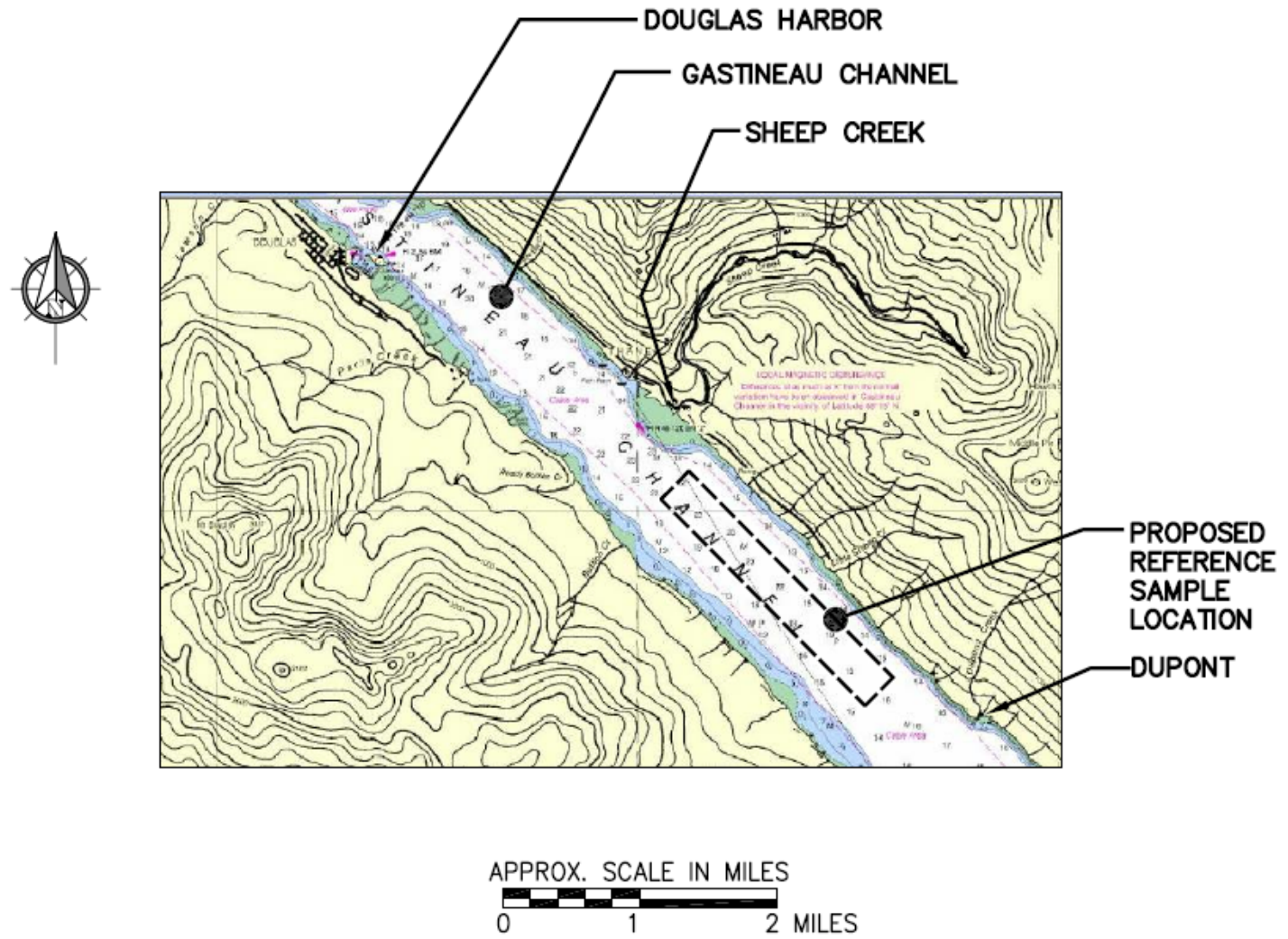


Figure 1-3. Nautical Chart of Reference Area

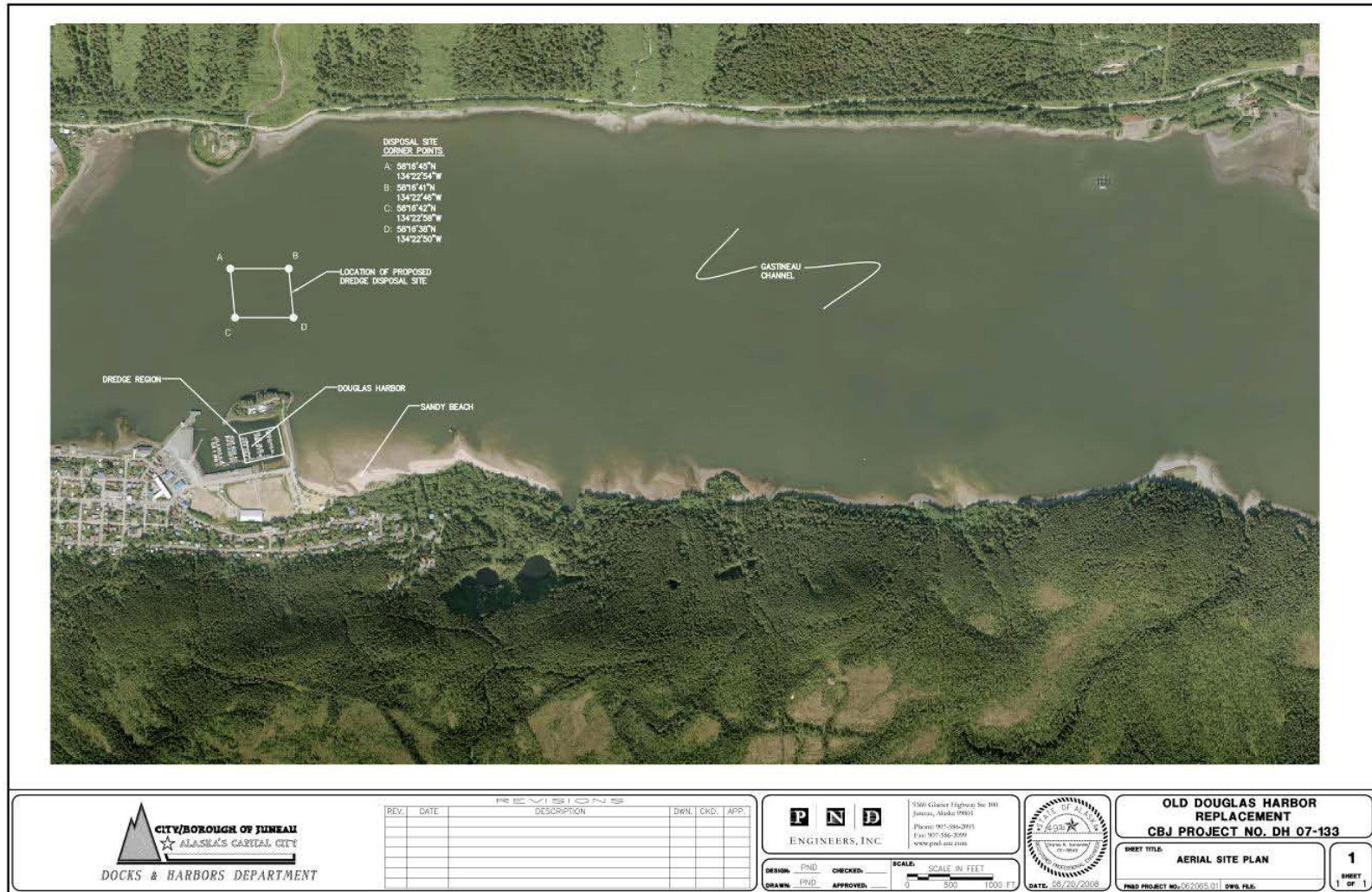


Figure 1-4. Aerial View of Douglas Harbor and the Proposed Disposal Site. *The reference area is not shown on this map.*

The five reference samples were treated as individual spatial replicates for biological testing and were submitted as individual samples for chemical analysis. These five reference samples were tested concurrently with the Douglas Harbor sediment treatments and the biological results were statistically compared to the test sediments. The comparison of reference and test sediment data provided a framework for determining suitability of the Douglas Harbor sediment for disposal at the GC site. Using the five spatial replicates in the comparison incorporates the inherent natural variability of the channel.

The five reference samples were also combined into one reference area composite based on guidance provided in the ITM when the disposal site is considered heterogeneous in nature (field investigation of the disposal site confirmed heterogeneity of disposal site, data provided in Appendix A to this report). This reference area approach is “used when the disposal site is known to be heterogeneous and more than one reference location must be sampled to adequately characterize the disposal site”.

1.3 STRATEGY FOR TESTING COMPOSITES AND STATION LOCATIONS

The estimated volume of Douglas Harbor dredged material is approximately 30,000 cy. Based on the project footprint, four area composites plus one lower composite were prepared and submitted for toxicological testing (Figure 1-5). This compositing scheme is consistent and more frequent than guidance provided in the ITM requiring a minimum of two sediment composites from eight sampling locations for volumes of 20,000-100,000 cy). The previous sediment investigation of Douglas Harbor identified four different dredged material management units (DMMU; the smallest volume of dredged material capable of being dredged independently from adjacent sediments) (PND 2007). Three of these DMMU areas (1, 2, and 4) are part of this investigation (for comparison to 2007 data the sample location names have not been changed and are shown in Figure 1-5). The sampling locations included the areas previous sampled in 2007 and a few new stations (NF prefix) to refine areas where sediment is currently accumulating.

Table 1-2. Number of Samples and Number of Composites per Dredge Volume.

Dredge Volume (cubic yards)	Number of Sampling Stations	Number of Composites
Recommended by ITM (USEPA/USACE 1998)		
5,000 – 20,000	4	1
20,000 – 100,000	8	2
100,000 – 200,000	12	3
Compositing Scheme for Douglas Harbor		
30,000	18	4

The sediment cores were opened and visually characterized prior to compositing. During this process, a change in the sediment type was observed based on depth of core with silty material in the upper layers and sandy material in the lower layers of each core. Vertical compositing was done to separate the upper and lower layers. Upper composites were kept distinct by area as designated on Figure 1-5, the lower composite represented the sandy material throughout the dredge footprint.

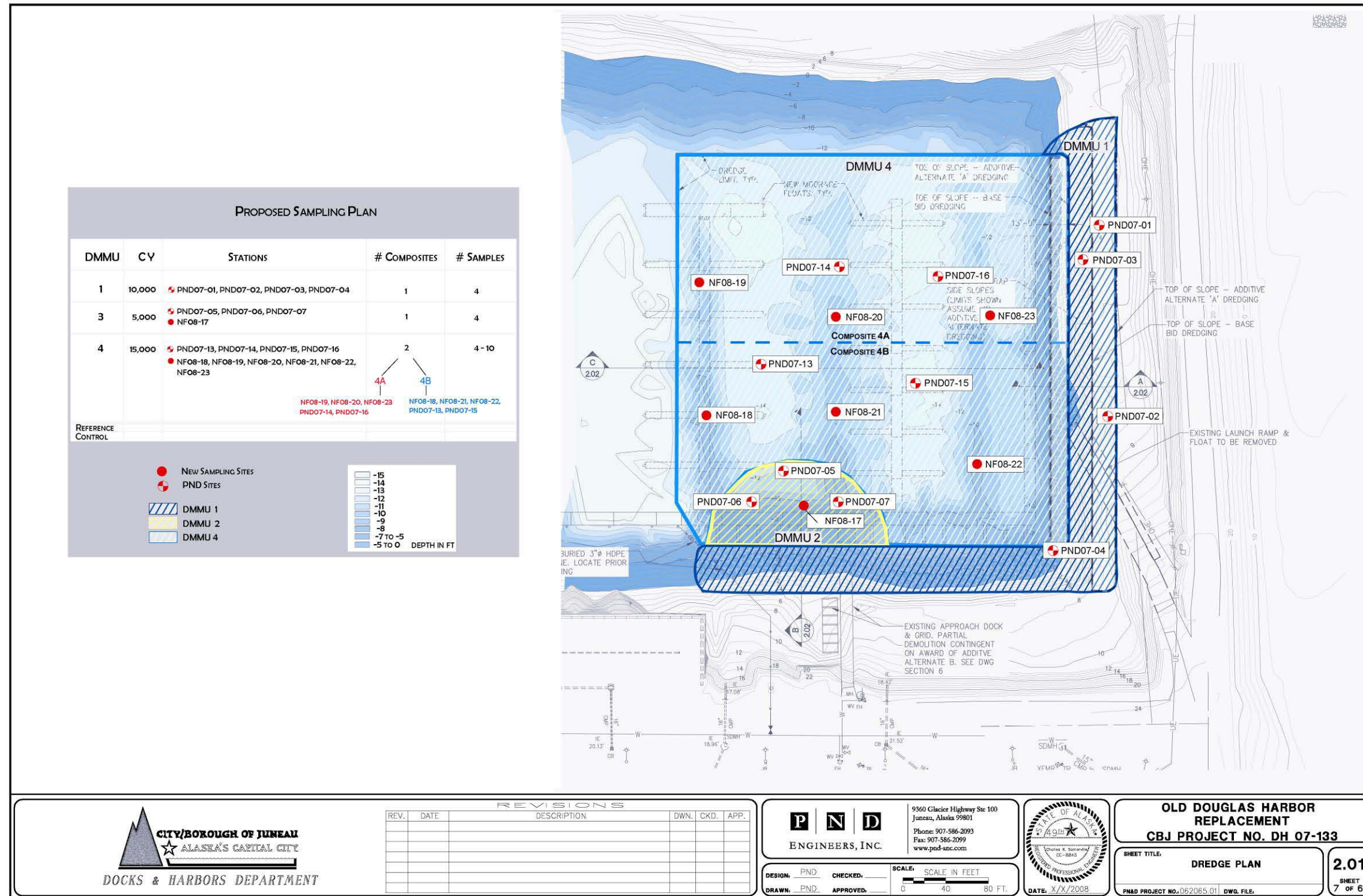


Figure 1-5. Douglas Harbor Site Map with Field Sampling Locations and Compositing Strategy.

The ITM (USEPA/USACE 1998) and the ITM Supplement (USEPA et al. 2001) provided guidance on compositing strategies:

- Combining locations from contiguous portions of the project area, using similar sediment types exposed to the same influences and pollutant sources.
- The amount of material taken from individual cores for allocation to the test composite was directly proportional to the length of core collected. The amount of test material required for each test composite (including sediment chemistry biological testing and bioaccumulation testing) was approximately ten gallons.
- The procedure for compositing included sediment from the entire length of core to project depth, however, because individual core samples contained distinct layers the core was split vertically to separate any effects that might occur from differing sediment types.

2 METHODS

2.1 FIELD SAMPLING AND SAMPLE COMPOSITING

Sediment cores were collected at eighteen stations; attempts were made to sample to a project depth of -14 ft MLLW at all locations. Table 3-1 located in the results section provides a summary of the data collected in the field.

Two sampling devices, a push core and a vibratory core, were used for the in-harbor sampling based on their ability to work in a variety of sediment types and water depths. These samplers were selected because they can collect the large sediment volumes necessary to accommodate both chemical and biological analyses.

A reference area approach was used for determination of suitability of the material for disposal. Individual reference sediment samples were collected from areas expected to be outside of the influence of the disposal site using a van Veen grab sampler. The exact locations of the reference sites were chosen in consultation with the regulatory agencies, PND, CBJ, and NewFields. The reference area samples and the reference area composite serve as a point of statistical comparison to the test data.

In addition to the reference area samples, van Veen grab samples were collected from several locations within the Gastineau Channel disposal site for characterization of the sediment type within the site.

2.1.1 CORE COLLECTION TECHNIQUES

All core sampling occurred onboard the tug vessel *WALDO* that has deck space and crane lifting capabilities to accommodate the field collection equipment. Figure 2-1 is a photograph of the vessel used for the inner harbor samples.



Figure 2-1. Waldo Vessel used for Sampling

The process for sediment collection was similar using either the push core or the vibratory hammer core except that the push core was manually pushed through the sediment and the vibratory core was vibrated through the sediment using a vibrating head. The procedure involved lowering the coring device to the sediment surface and then driving the core through the sediment to project depth. When the sampler could not penetrate to project depth due to the sediment type, the vessel was moved and a second attempt was made to collect a sample. Individual Lexan[®] core liners were used inside the core tube with separate liners used for each station. Once onboard the vessel, the core was placed horizontally on the deck and the core liner was extruded, cut into smaller sections, capped on either end, and placed in coolers containing blue ice to provide storage at temperatures of approximately 4°C.

2.1.2 VAN VEEN GRAB COLLECTION

A stainless steel van Veen grab sampler was used to collect the reference sediment samples. The R/V Summer King was used for transit to the proposed Gastineau Channel reference area. Sediment representing the upper 10 - 12 centimeters within a sampling area of 0.1 square meters was collected and transferred to labeled polyethylene bags and stored in coolers maintained at approximately 4°C during all aspects of shipping and handling. Approximately five gallons of sediment per station sample was taken, which required two to five grabs samples per site.

2.1.3 WATER COLLECTION FOR WATER COLUMN TEST PREPARATION

Douglas Harbor site water was collected into pre-cleaned polycarbonate carboys. A clean, hand operated piston type pump, was placed below the water surface and water was pumped into the clean carboy. This procedure avoids collecting any surface water that may contain oil or other materials that could interfere with the test. Approximately 120 L of site water was collected to conduct the three water column tests using a clean water pump submerged just below the water surface inside the harbor.

2.1.4 NAVIGATION

All station locations were determined using a Differential Global Positioning System (DGPS). The system uses U.S. Coast Guard differential correction data, and is accurate to ± 3 meters. All final station locations were recorded in the field using positions from the DGPS.

2.1.5 SEDIMENT HANDLING

The core stratigraphy was recorded in the field log by viewing through the clear Lexan[®] core liner. The core was cut into two to three foot sections and placed into labeled coolers maintained at approximately 4° C until delivery to the NewFields' laboratory in Port Gamble, Washington for processing. Upon return to NewFields in Port Gamble, a representative core from each station was photographed and characterized for sediment characteristics. The geologic description of each core included the texture, odor, color, length, approximate grain size distribution, and any evident stratification of the sediment. All field sampling and core processing data are summarized in Appendix A.

When the sediment cores were composed of different sediment types they were segregated into different vertical composites. The upper composites were representative of the four DMMUs discussed in Section 1.3, the lower portion of the cores were mixed into one composite representing the entire dredge footprint. Adequate sediment was collected to perform additional chemical and biological analysis, if necessary.

Sediment collected from the reference sites was placed into clean, polyethylene bags, labeled (project name, date, sampler ID), logged into a field chain-of-custody (COC) form, and placed into a cooler maintained at approximately 4° C until delivery to the NewFields' laboratory in Port Gamble, Washington for processing.

Every cooler contained a temperature blank that is used to assess the temperature of the cooler upon arrival at the testing laboratory and a chain of custody form was attached to the inside of the cooler lid.

2.1.6 SAMPLE PROCESSING AND STORAGE

Sample processing and composting was performed at the Port Gamble NewFields laboratory. Each sediment sample was homogenized to a uniform consistency at the laboratory using a stainless steel mixing bowl and spoon. Each test composite was generated by allocating sediment from each station based on the length of core collected.

Samples for physical and chemical analysis were placed into certified clean glass jars with Teflon-lined lids and shipped to the analytical laboratories. Sub-samples for archive were placed in certified clean glass jars with Teflon-lined lids and frozen at -20°C for possible future chemical analysis in the event that further delineation of chemical contamination among stations is required. The remainder of the composite sample was analyzed for toxicity and bioaccumulation potential. All sediment samples were stored in the walk-in cold room at the Port Gamble laboratory maintained at a constant temperature of approximately 4°C.

2.1.7 SHIPPING

Chemistry jars for mercury analysis were provided by the analytical laboratory (Battelle Marine Sciences Laboratory). The analysis jars were cleaned according to methods outlined for mercury analysis. Briefly, the cleaning process involved washing the bottles or glass jars and then boiling them in concentrated HNO₃ for 48 hours. Bottles were then rinsed in tap water shown to contain negligible concentrations of methyl mercury, and then filled with 0.5% HCl in low Hg water and heated to 65°C for a minimum of 24 hours. This solution was then poured off and the bottles were refilled with 0.5% HCl in low Hg water, and then stored until use. Prior to use, the vessels were emptied and dried in a clean drying oven at 65°C.

After the sediment was composited and sampled for chemical analysis, the chemistry sample jars were placed in sealable plastic bags and securely packed inside a cooler with blue ice. The COC forms were completed and the original signed COC forms were placed in a sealable plastic bag and placed inside the cooler. The cooler lids were securely taped shut.

2.2 DECONTAMINATION OF FIELD AND LABORATORY EQUIPMENT

All sampling and laboratory equipment were cleaned prior to sampling. In the field the core and grab samplers were rinsed between stations with site water. To avoid cross contamination between stations, individual core Lexan[®] liners were used to collect the sediment samples.

Sediment composting was conducted at the Port Gamble laboratory using clean sampling techniques. All stainless steel utensils (bowls, spoons, spatulas, mixers, and other utensils) were cleaned with soapy water, rinsed with tap water, and then rinsed three times with deionized water. The final cleaning step was a rinse with acetone to remove any trace of soap or organic residue. Glassware was cleaned with soapy water, rinsed with deionized water, soaked in a hydrochloric

acid bath and rinsed with acetone prior to use. After the acetone rinse the item was rinsed in deionized water again.

2.3 DOCUMENTATION AND CHAIN OF CUSTODY

Samples were considered to be in custody if they were: (1) in the custodian's possession or view, (2) retained in a secured place (under lock) with restricted access, or (3) placed in a secured container. The principal documents used to identify samples and to document possession were COC records, field logbooks, and field tracking forms. COC procedures were used for all samples throughout the collection, transport, and analytical process, and for all data and data documentation, whether in hard copy or electronic format.

The COC procedures began during sample collection. A COC record was prepared for each sample. Each person who had custody of the samples signed the form and ensured that the samples were properly secured. Minimum documentation of sample handling and custody included the following:

- Sample identification
- Sample collection date and time
- Any special notations on sample characteristics
- Initials of the person collecting the sample
- Date the sample was sent to the laboratory
- Shipping company and waybill information

2.4 PHYSICAL AND CHEMICAL ANALYSIS

Physical and chemical parameters measured in sediment for this testing program were selected to provide confirmatory data on potential chemicals of concern in the dredged material from Douglas Harbor in accordance with the ITM (USEPA/USACE 1998). Test and reference sediments were analyzed for the parameters and target detection limits indicated in Table 2-2. All analytical methods used to obtain contaminant concentrations followed EPA or Standard Methods.

2.4.1 PHYSICAL ANALYSES

To characterize the physical properties of the sediment, tests were performed to predict the behavior of sediment after disposal and to compare reference and test sediment. Physical-chemical analyses of the sediment included grain size, total organic carbon (TOC), and total solids. Grain size determines the general size classes that make up the sediment (e.g., gravel, sand, silt, and clay). The frequency distributions of the size classes (reported in millimeters [mm]) of the sediment are reported in Appendix B.

Grain size was conducted using the gravimetric procedure described in Plumb (1981). Total organic carbon (TOC), made up of volatile and nonvolatile organic compounds, was determined as recommended in the ITM (USEPA/USACE 1998) or equivalent (modified SW846). This procedure involved dissolving inorganic carbon (carbonates and bicarbonates) with hydrochloric acid or sulfuric acid prior to TOC analysis (Plumb 1981). Total solids were measured to convert concentrations of the chemical parameters from a wet-weight to a dry-weight basis. Percent solid measurements were determined by USEPA Method 160.3 (USEPA 2001).

Acid Volatile Sulfide (AVS) and Simultaneously Extracted Metals (SEM) in sediment followed the published procedure (Allen et al. 1991) for the analysis of acid volatile sulfide (AVS) in sediment

and total sulfide in aqueous samples. For sediment samples, sulfide was volatilized after the addition of acid. The acid extraction produced in this step was also analyzed for simultaneously extractable metals (SEM) that became soluble during the acidification step. As a precipitant with heavy metals, sulfide is fundamental in the determination of the bioavailability of metals in anoxic sediment. When the molar ratio of SEM to AVS exceeds one, the metals are potentially bioavailable to aquatic organisms.

Table 2-1. Physical and Chemical Measurements, Analytical Methods, and Detection Limits

Parameter	Method	Procedure	Sediment Reporting Limit (dry weight)	Water Reporting Limit	Tissue Reporting Limit (wet weight)
Grain Size	Plumb (1981)	Sieve/Pipette	1.0%		
Total Organic Carbon	ASTM D2579	Combustion IR	0.1%		
Percent Solids	EPA 160.3	Gravimetric	0.1%		
AVS/SEM	Allen et al 1991	ICP-MS	AVS: 0.0119 $\mu\text{mole/g}$ Cd: 0.0000661 $\mu\text{mole/g}$ Cu: 0.00257 $\mu\text{mole/g}$ Ni: 0.000512 $\mu\text{mole/g}$ Pb: 0.0000359 $\mu\text{mole/g}$ Zn: 0.000795 $\mu\text{mole/g}$ Hg: 0.000000278 $\mu\text{mole/g}$		
Ammonia	Standard Methods 4500 NH ₃ D ;ASTM Method D 1426-93 Test Method B; and USEPA Method 350.3	Ion Selective Method		0.5 mg/L	
Lipids	Bligh Dyer	Gravimetric			0.1%
Total Mercury (Hg) sediment and tissue	USEPA 7473	CVAA	0.002 $\mu\text{g/g}$		0.002 $\mu\text{g/g}$
Total Mercury (Hg) water	USEPA 1631	CVAF		0.2 (ng/l)	
Methyl Mercury (Hg) sediment, water	USEPA 1630	CVAF	0.00002 $\mu\text{g/g}$	0.03 (ng/l)	

Acid volatile sulfides analysis used a colorimetric method in which the sulfide in the sample was converted to hydrogen sulfide by the addition of hydrochloric acid at room temperature. The hydrogen sulfide (H₂S) was purged from the sample by an inert gas and trapped in a sodium hydroxide (NaOH) solution. With the addition of a mixed-diamine reagent (MDR), the sulfide was converted to methylene blue and measured on a spectrometer. The acid-sediment slurry was decanted into a centrifuge tube and centrifuged to settle the sediment. The supernatant was poured

into an acid cleaned Teflon bottle, ready to be analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), silver (Ag) or zinc (Zn) following a modification of EPA Method 1638; and by Cold Vapor Atomic Fluorescence (CVAF) for Hg following EPA Method 1631.

Ammonia was measured in the overlying water and in the pore water of the biological tests following methods referenced in Table 2-2.

2.4.2 METHYL MERCURY IN WATER AND SEDIMENT

The method used for methyl mercury (Hg) followed Bloom (1989) for the determination of methyl mercury in a wide range of biological and geological matrices. This CVAF technique operated in the emission of 254 nm radiation by excited Hg atoms in an inert gas stream. This method is currently contained in 1600 series for trace metals analysis (EPA Method 1630).

Sediment and pore water samples were distilled in Teflon vessels using the methods of Horvat et al. (1993). Alternatively, sediment samples can also be prepared for analysis using the method of Bloom et al. (1997). This new extraction technique avoids the methylation artifact sometimes produced in sediment sample containing high levels of inorganic mercury and organic carbon. An ethylating agent was added to the digestate or distillate to form a volatile methyl-ethyl mercury derivative and the derivative was purged onto graphitized carbon traps as a means of pre-concentration and interference removal. The mercury species were separated using isothermal chromatography, broken down to elemental mercury by means of pyrolysis, and detected using a CVAF detector as described in Bloom and Fitzgerald (1988). The detection limits were 0.00002 µg/g for sediment (0.02 ppb), and 0.03 ng/l (0.03 ppt) for water.

2.4.3 TOTAL MERCURY IN WATER

EPA Method 1631 is used routinely for the analysis of total mercury in water. This method uses a CVAF technique, based on the fluorescence of excited Hg atoms in an inert gas stream at 254 nm wavelength (Bloom and Creclius 1983). To determine total mercury, water samples were oxidized with bromine monochloride, which breaks down organo-mercury bonds. Mercuric ions in the oxidized sample were reduced to Hg with SnCl₂, and then purged onto a gold trap as a means of pre-concentration and interference removal. Mercury vapor was thermally desorbed into the fluorescence pathway. Fluorescence (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged. Typical detection limit for total mercury reported as 0.2 ng/l as Hg or 0.2 parts per trillion.

2.4.4 TOTAL MERCURY IN SEDIMENT AND TISSUE

The analysis of total mercury in sediment employs a CVAA technique based on the absorption of 254 nm radiation by excited Hg atoms in an inert gas stream. To determine total mercury, a known mass of each sample was combusted at 750°C. The evolved Hg ions were then swept into the absorption pathway. Absorption (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged. This method quantifies all mercury in the sediment including lithologic mercury. The typical detection limit for the method was 0.002 µg/g as Hg.

2.4.5 BIOACCUMULATION TISSUE CHEMISTRY

Total mercury analysis of tissues was performed to demonstrate the availability of sediment contaminants for accumulation by test organisms. Tissue composites from each replicate were analyzed separately.

2.5 BIOASSAY TESTING

Samples were evaluated in accordance with procedures outlined in the ITM (USEPA/USACE 1998) to establish suitability of sediment for disposal of dredged material in inland waters. This program included bioassay analysis of four area composite samples and two reference samples (a reference composite and one reference sample (REF X) comprised of five reference samples as independent replicates. In addition, appropriate laboratory control samples were run with each of the selected test species. Ammonia concentrations in composite sample pore-water were analyzed prior to bioassay testing in the bulk sediments. Bioassay testing for this project consists of two benthic toxicity tests, three water-column (WC) toxicity tests, and two bioaccumulation potential (BP) tests. The bioassays conducted in support of this project are summarized in Table 2-2.

Table 2-2. Biological Testing Performed for Dredged Material Evaluation.

Test Type	Type of Organism	Taxon	Project Sediments	Control Sediment/ Seawater	Reference ¹ Toxicant
Benthic	Polychaete	<i>Neanthes arenaceodentata</i>	X	X	X
	Amphipod	<i>Ampelisca abdita</i>	X	X	X
Water-Column	Fish	<i>Menidia beryllina</i>	X ²	X	X
	Mysid	<i>Americamysis bahia</i>	X ²	X	X
	Bivalve larvae	<i>Mytilus sp.</i>	X ²	X	X
Bioaccumulation Potential	Bivalve	<i>Macoma nasuta</i>	X	X	
	Polychaete	<i>Nephtys caecoides</i>	X	X	

¹Shaded areas indicate tests or treatments that are not applicable to the selected tests.

² Sediment elutriates of project material

2.5.1 BENTHIC TESTS

Benthic tests were performed to estimate the potential impact of inland water disposal of dredged material on benthic organisms that attempt to re-colonize the area. Sediment was tested using two species: the polychaete *Neanthes arenaceodentata* and the amphipod, *Ampelisca abdita*. Species of these two genera are typical inhabitants of Alaska subtidal sediments.

Juvenile polychaete worms (*N. arenaceodentata*) were supplied by Donald Reish, Ph.D., Long Beach, California. Juvenile polychaetes were held in seawater at 20°C (*Neanthes* are cultured in water-only and are not held in sediment prior to testing). Control sediment used in the benthic polychaete test was sediment from Yaquina Bay, Oregon; this sediment is native sediment supplied with the amphipod *Eohaustorius estuarius* and typically used by NewFields for control sediment in *N. arenaceodentata* testing.

Ampelisca abdita were obtained from John Brezina in Tomales Bay, California. Organisms were held at 20°C prior to testing. Native sediment was also provided and used as control sediment in the amphipod test.

Test organisms were exposed to the sediment for ten days in 1-liter glass test chambers. Two centimeters of sediment (approximately 150 mL) were placed into each chamber with 800 mL of overlying water. The bioassays were performed as static tests with no feeding during the exposure period. Initial stocking densities in each replicate were 20 organisms per test chamber for the amphipod test, and 5 organisms per test chamber for the polychaete test. Trickle-flow aeration was provided through glass pipettes, and care was taken to avoid disturbing the sediment surface. Water quality measurements were taken in one replicate for each test treatment daily and included pH, salinity, temperature, and dissolved oxygen. Ammonia was measured in both interstitial (pore water) and overlying water at the start and finish of the test from a surrogate chamber for each test treatment. Sediment pore water was extracted via centrifugation. All instruments were calibrated and logged daily. At termination, the sediments were carefully sieved to remove the test organisms and survivorship assessed using methods described in the ITM (USEPA/USACE 1998). To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980).

2.5.2 WATER-COLUMN TESTING

Water-column tests were performed to estimate the potential impact of dredged material to organisms that live in the water column. The WC test was performed using a 4:1 dilution by volume of site water to sediment. Sediment from each composite was combined with water collected from the project site, vigorously agitated for 30 minutes, and then centrifuged for approximately 30 minutes at room temperature (16–18°C). Following centrifugation, the supernatant was gently decanted. This supernatant represents the 100% test concentration and was used to create serial dilutions with clean seawater (0.45- μ m filtered Hood Canal seawater) to create subsequent test concentrations for the water-column tests. Three species were tested: *Mytilus sp.* (Bivalve larvae), *Americamysis* (formerly *Mysidopsis*) *bahia* (mysid shrimp), and *Menidia beryllina* (inland silverside fish).

The bivalve larval test was run on the test dredged material elutriates at 100%, 50%, 10%, and 1% dilutions, a clean seawater control and a site water control. There were five replicates per elutriate. The test exposure was approximately 74 hours to ensure development of the bivalve larvae to the D-hinge stage in the control. At the termination of the study, survival and normal development were compared between the control and test groups to determine if significant mortality or abnormal development occurred. The percent normal development of the test treatments were normalized for control responses.

For *A. bahia* and *M. beryllina*, the WC test was performed with dilutions of 100%, 50%, and 10% of elutriate as well as a clean seawater control and site water control under static conditions. Ten animals were used per replicate with five replicates per elutriate concentration. These tests were run for 96 hours.

Daily water quality monitoring of test chambers was carried out for pH, dissolved oxygen, salinity, and temperature. Ammonia was analyzed at the start and end of the tests in all concentrations. To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980).

2.6 ACCLIMATION OF TEST SEDIMENT

Additional testing was conducted to address acclimation of sediment to testing conditions. The acclimation efforts focused on two test composites (Area 4B based on high pore water ammonia and Lower Comp based on physical characteristics of the sediment) and the reference composite. Acclimation was required because additional contributions to toxicity may have been related to the changes in microbial processes that occur when sediment is placed into conditions established for toxicity testing that are different from conditions where the sediment was collected. Sediment such as the Lower Comp that has been deeply buried and isolated from biogenic processes (deeper than 10 cm below mud line depths) and any sediment composites that have pore water ammonia values above threshold levels eliciting a negative response in test species, need to be exposed to test conditions to allow the naturally occurring contributory factors to dissipate.

The amount of time required for acclimation is dependent on the water quality parameters of the sediment. Sediment taken from one environmental regime to another (e.g., fresh water to marine or from deep non-biogenic materials to biogenic surface material) undergoes natural microbial changes to accommodate to the new environment. A surrogate measure of the success of this process was to measure the overlying water ammonia concentration through time. The premise for using ammonia as a surrogate assumes that ammonia concentrations increase until the microbial community adjusts to the new environment. Once the microbial community was established, the overlying water ammonia concentration decreased to levels below species-specific threshold concentrations. Although, ammonia is a surrogate measure to indicate when the acclimation process was complete, acclimation of test sediment addresses other potential contributing factors including sulfide toxicity.

The differences in survival of test organisms between acclimated and unacclimated testing are attributed to the acclimation process. The premise of acclimation is that effects from the acclimated sediment represents contaminant related effects, effects from unacclimated sediment represent contributions from contaminants as well as other more transitory effects that are observed when changes occur in the biogenic nature of the sediment.

The acclimation process was performed on an additional five replicates of each test composite sample and the reference composite samples. The testing on the acclimated sediment was conducted at the same time as the standardized tests. The only difference was the period of time that the sediment was exposed to seawater before the test organisms are added to the sediment treatments. In the standard tests, sediment was exposed to seawater for one day prior to the addition of test organisms to the test containers; the acclimated sediment was exposed to seawater for approximately one week prior to the addition of test organisms.

2.6.1 BIOACCUMULATION POTENTIAL TESTING

Assessment of bioaccumulation potential was carried out using the polychaete worm *Nephtys caecoides* and the bivalve *Macoma nasuta* over a 28-day test period. Bioaccumulation tests were conducted in accordance with those procedures outlined in *Guidance Manual: Bedded Sediment Bioaccumulation Tests* (USEPA 1993) and Appendix E of the ITM (USEPA/USACE 1998). Each of these tests was initiated using test, reference, and control sediments. Five replicate tests were performed for each composite sample. *N. caecoides* exposures were conducted using 25 animals in each of five replicate test chambers. For *M. nasuta* exposures, 10 animals were placed in each of five replicate test chambers. The test chambers were maintained under flow-through conditions, and daily water quality measurements were recorded for each chamber. On Day 28, the sediment was sieved to remove the worms and clams. The surviving *M. nasuta* and *N. caecoides* were placed

in clean flow-through aquaria to purge their gut contents over 24 hours, and then tissues were placed into certified-clean glass sample jars, frozen and sent to the chemistry laboratory for tissue analysis. In order for the *N. caecoides* to purge their gut content, clean sand was also added to the clean aquaria.

The physical characteristics of the Lower Comp treatment included silty-sand sediment with very low total organic carbon content. This composite was acclimated, prior to test initiation, with raw sea water to encourage microbial growth to provide a food source for the test organism throughout the duration of the testing. The raw seawater was statically renewed daily until the start of the test and ammonia was monitored in the overlying water. One day before the start of the bioaccumulation test, the Lower Comp treatments were converted from raw seawater to filtered flowing seawater to match the set up of the other test treatments.

2.6.2 SEAWATER FOR BIOASSAY TESTING

Seawater used in this study, including the flow-through studies, came from the Hood Canal at Port Gamble, Washington. This seawater source has been used successfully on similar bioassay testing programs by the contracting team. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation from this water supply. Good survival of organisms in control sediment has been achieved consistently in previous dredge material testing conducted by the laboratory and the site is also being used to produce larval seed organisms for aquaculture purposes.

2.7 DATA MANAGEMENT AND ANALYSIS

All water quality and endpoint data were entered into Excel spreadsheets. Water quality parameters were summarized by calculating the mean, minimum, and maximum values for each test treatment. Endpoint data were calculated for each replicate and the mean value and standard deviation were determined for each test treatment.

All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the ITM (USACE/USEPA 1998) and, where appropriate, Puget Sound Dredged Material Evaluation and Disposal Procedures (USACE 2008). All statistical comparisons were performed using SAS/STAT® software (SAS Institute 2007).

All data were tested for the assumptions of normal distribution and equality of variance prior to statistical comparisons. The Shapiro-Wilk's test was used to test for normal distribution ($\alpha=0.01$, $N>20$, balanced design) and the Levene's test was performed to test for equality of variance ($\alpha=0.10$, $n=5$, balanced data).

Water column data were tested with one-tailed t-tests on arcsine-square root transformed data. Data with equal variances were compared using the combined variance; those with unequal variances used the Satterhwaite approximation for computing the test statistic. When data were not normally distributed, the t-test was performed on rankits transformed data.

Benthic survival data were tested according to both the PSSDA (USACE 2008) and ITM (USACE/USEPA 1998) methods, using arcsine-square root transformed data. PSSDA statistical

guidance calls for one-tailed t-tests on normally distributed data with either the pooled variance (equal variances) or Satterthwaite approximation (unequal variances). When data were not normally distributed, the t-test was performed on rankits transformed data. For the ITM statistics, when data met the assumptions of normality and equality of variance, an Analysis of Variance (ANOVA) with a Fisher's least significant difference (LSD) comparison on the means (one-tailed, $\alpha=0.05$) was performed. Data with normal distributions and unequal variances were tested with a one-tailed t-test (the same test as performed for PSSDA).

Concentrations of mercury in tissues exposed to test composite samples were compared to the reference composite concentrations following guidelines in the ITM (USACE 1998). All concentrations were above detection limits; therefore no censored data application was needed. When untransformed data did not meet the assumptions of normality or equal variance, the data were transformed with a natural log and retested. Data meeting both assumptions were tested with an ANOVA with a LSD comparison on the means (one-tailed, $\alpha=0.05$). When data were normally distributed but variances were unequal, individual comparisons of each test composite to the reference composite were made with a t-test using the Satterthwaite approximation for a test with unequal variance.

Comparisons of the tissue test composites were also made to the action level for mercury (0.32 ppm) requested by ADEC to address potential human health concerns and to the ERED database (USACE/USEPA 2008) maintained by the USACE – ERDC to evaluate potential ecological risk. For these comparisons, the 95% upper confidence limit on each tissue composite was calculated using the mean square error from the ANOVA when variances were equal or the variance for the sample when variances were unequal. Calculations were performed on log transformed data as appropriate and the results back-transformed for comparison to the action level.

2.8 QUALITY ASSURANCE/QUALITY CONTROL

2.8.1 FIELD SAMPLING QA/QC

Field sampling data were assessed on comparability, representativeness, and completeness. Accuracy and precision of field data were achieved by use of standardized methods of locating sampling points such as differential Global Positioning Systems, with visual verification to known landmarks. Comparability and representativeness for field sampling were achieved by use of standardized sampling equipment appropriate for the sampling location.

Field logbooks provide documentation of all sample collection activities performed. Entries were described in as much detail as possible so that persons going to the project site could reconstruct a particular sampling event. At the beginning of each field day, the date, start time, weather, names of sampling and/or investigative personnel present, were entered and signed by the person making the entry.

Information on sample collection was recorded in the logbook. All entries were made in ink. If an incorrect entry was made, the information was crossed out with a single strike mark. Wherever a sample was collected or a measurement was made, a detailed description of the location, with relevant information such that the sampling point can be relocated or mapped at a later time. Location information included GPS coordinates; any appropriate reference points and distance measurements. Any photographs taken of the station were documented. Equipment used to make field measurements were identified, along with the date of calibration.

A description of the equipment used to collect samples was entered, along with the date and time of collection, sample description, depth from which sample was collected, volume and number of containers. Sample identification numbers were assigned during sample collection. Duplicate samples received a separate sample number and were noted under the sample description.

Sample containers were provided by the analytical laboratory, who maintain documentation of the manufacturer, grade, lot number and/or other identifying information regarding preservatives added to sample containers. Chain-of-custody forms were maintained for each sample collected.

2.8.2 ANALYTICAL CHEMISTRY QA/QC

Table 2-4 lists specific data quality objectives for each group of analyses performed. The parameters used to assess data quality were precision, accuracy, representativeness, comparability, and completeness.

Table 2-3. Data Quality Objectives for Mercury Analysis

QC Measurement	Frequency	Acceptable Limits	Corrective Action
Total Mercury in Sediment and Tissue			
Method blank	1 per ≤20 samples	< 5 times the MDL	Reanalyze. If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the batch must be reanalyzed
Certified/Standard Reference Samples	1 per ≤20 samples	80-120% of certified value	Reanalyze. Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Matrix Spike	1 per ≤20 samples	80 – 120% recovery	Reanalyze. Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Replicate Precision	1 per ≤20 samples	20% for analytes > 3 times the MDL. No more than 35% of all RPDs can be >25%	Reanalyze. Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Initial and Continuing Calibration Verification	Every 10 samples	10%/20% of initial calibration	Reanalyze. If subsequent ICV or CCV still fail, rerun the calibration curve and all samples analyzed after the last passing calibration check.
Total Mercury in Aqueous Samples			
Method blank	1 per ≤20 samples	< 5 times the MDL	If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the bath must be reanalyzed
Certified/Standard Reference Samples	1 per ≤20 samples	77-123 % of certified value	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.

QC Measurement	Frequency	Acceptable Limits	Corrective Action
Matrix Spike	1 per ≤20 samples	71- 125 % recovery	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Replicate Precision	1 per ≤20 samples	21% for analytes > 3 times the MDL. No more than 35% of all RPDs can be >21%	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Initial and Continuing Calibration Verification	Every 10 samples	<15% of initial calibration	If subsequent ICV or CCV still fail, rerun the calibration curve and all samples analyzed after the last passing calibration check.
Methyl Mercury in Sediment, and Aqueous Samples			
Method blank	1 per ≤20 samples	< 5 times the MDL	If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the bath must be reanalyzed
Certified/Standard Reference Samples	1 per ≤20 samples	66-123 % of certified value	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Matrix Spike	1 per ≤20 samples	65- 135 % recovery	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Replicate Precision	1 per ≤20 samples	35% for analytes > 5 times the MDL. No more than 35% of all RSDs can be >35%	Failure to meet the acceptable limits shall be reported in the Data Summary. Failure of multiple data quality objectives requires the samples to be reanalyzed.
Initial and Continuing Calibration Verification	Every 10 samples	<20% of initial calibration	If subsequent ICV or CCV still fail, rerun the calibration curve and all samples analyzed after the last passing calibration check.

The QA objective with respect to accuracy, precision, and sensitivity of laboratory data was to achieve the QC acceptance criteria of the testing protocols. In general, the accuracy and precision criteria were stipulated by the most recent versions or modifications of USEPA SW-846.

To assess the quality of data resulting from the analytical chemistry program, the following QA/QC measures were included in the sampling program:

- Procedural blanks were performed to check for artifacts associated with sample extraction and analysis. Procedural blanks will be performed at a rate of one per 20 samples or each analytical batch.
- Sufficient sample volume was supplied to the laboratory in order to perform matrix spike/matrix spike duplicate (MS/MSD). MS/MSD samples evaluated analytical accuracy and precision. MS/MSD samples were performed at a frequency of one per 20 (5%) investigative samples or each analytical batch.

- Laboratory duplicate sample analyses were performed to check precision of the analytical process. Lab duplicate samples were conducted at a frequency of one per 20 (5%) investigative samples or one per analytical batch.
- A standard reference material analysis was conducted when appropriate to evaluate the analytical accuracy. An SRM analysis was conducted at a frequency of one per 20 samples (5%) or one per analytical batch.

2.8.3 TOXICITY TESTING QA/QC

The quality assurance objectives for toxicity testing conducted by the testing laboratory are provided in detail in the ITM (USEPA/USACE 1998). These objectives for accuracy and precision involve all aspects of the testing process, included the following:

- Water and sediment sampling and handling
- Source and condition of test organisms
- Condition of equipment
- Test conditions
- Instrument calibration
- Use of reference toxicants
- Record keeping
- Data evaluation

The sensitivity of the test organisms relative to established laboratory control charts was evaluated using reference toxicant tests. The reference toxicant LC₅₀ or EC₅₀ should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits and corrective actions were taken if necessary. All limits established for this program met or are more stringent than those recommended by USEPA.

All data collected and produced were recorded on approved data sheets and became part of the permanent data record of the program. If any aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

There is no established accuracy or precision requirement for toxicity tests. Acceptable accuracy levels were generally assessed by the calibration of water quality instruments, the use of certified standards, and the establishment of acceptable water quality testing parameters. For example, water quality was monitored and, adjusted if necessary, throughout testing in at least one test replicate. Parameters that fell outside of acceptable test ranges required corrective action. Deviations from water quality testing ranges do not necessarily fail the test; however, the potential impact on test exposures was discussed.

Test organism behavior was visually monitored for each test chamber. The system was evaluated by conducting concurrent tests with negative control sediment. Adequate organism survival, as specified in the ITM (USEPA/USACE 1998), indicated a healthy testing population. Control survival for test validation was species and method specific.

To ensure that each test chamber contains the appropriate number of test organisms, a second technician checked the number of organisms in each transfer cup prior to placement in the test chamber. Duplicate counts were performed at test termination. Random allocation of test organisms and testing chambers was conducted to remove any bias associated with selectively picking the strongest organisms first or any bias associated with location of test chambers.

Representativeness was maintained during toxicity testing by ensuring that sediment was held in the dark at 4°C until needed for testing. Test sediment homogenization occurred prior to placement in test chambers. All test chambers and utensils were washed in warm soapy water, DI rinsed, acid-rinsed, and solvent rinsed. Water quality parameters were measured daily in at least one replicate per treatment. A calibration check was performed daily on all water quality instruments.

The QA objective for comparability was used to make valid comparisons with data that may be generated in the future from the project site. This objective involved the analysis of environmental samples collected during the sampling program in a manner that produced results comparable to results that would be obtained by another laboratory using the same procedures. Comparability of the data was assessed by the use of standard materials traceable to the National Institute of Standards and Technology (NIST), or approved suppliers, such as established vendors for the purchase of test organisms, the use of a positive control for toxicity tests, the use of standardized, regulatory approved procedures for sample collection and sample analysis, and analysis of quality control samples to validate the analytical results.

Each test organism batch was evaluated in reference toxicant tests during the test period to establish the sensitivity of the test organisms. The reference toxicant LC₅₀ or EC₅₀ should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

The methods employed in every phase of the toxicity testing program are detailed in the NewFields Standard Operating Practices (SOP). All NewFields staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced were recorded on approved data sheets. If an aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

The test performance criteria followed the guidance described in the ITM (USEPA/USACE1998) Section 6.1 – 6.3. The performance criteria for this project were taken *directly* from the ITM:

WATER- COLUMN TESTING PERFORMANCE CRITERIA (ITM ONLY):

- The 100% dredged material elutriate toxicity is not statistically higher than the dilution water 0%, the dredged material is not predicted to be acutely toxic to water-column organisms.
- The concentration of dissolved and suspended contaminants, after allowance for initial mixing, does not exceed 0.01 of the toxic concentration expressed as the EC or LC₅₀, beyond the boundaries of the mixing zone. Therefore the dredged material is predicted not to be acutely toxic to water column organisms. However, benthic impacts have to be considered. If information warrants, it is acceptable to determine water column effects at Tier III and benthic effects at another tier.
- The concentration of dissolved plus suspended contaminants, after allowance for mixing, exceeds 0.01 of the toxic (LC or EC₅₀) concentration beyond the boundaries of

the mixing zone. Therefore, the dredged material is predicted to be acutely toxic to water column organisms.

Water-column tests are not routinely conducted as part of the Dredged Material Evaluation and Disposal Procedures (Users Manual) (USACE 2008), therefore interpretative criteria for the water-column test will follow guidance in ITM.

BENTHIC TOXICITY TESTING PERFORMANCE CRITERIA

ITM Performance Criteria for benthic tests were predicted to be acutely toxic to benthic organisms when mean test organism mortality:

- Is statistically greater than in the reference sediment **and**
- Exceeds mortality in the reference sediment by at least 10% (...20% value for lethality can be used for amphipods, *Ampelisca abdita*, *Rhepoxynius abronius*, or *Eohaustorius estuarius* (Swartz et al, 1985; Mearns et al., 1986; SAIC, 1992 a,b).

Interpretative Criteria for the amphipod test based on the Dredged Material Evaluation and Disposal Procedures (Users Manual) (July 2008):

- Mean test mortality is significant if it is greater than 20% (absolute) over the mean negative control response, and mean test mortality is greater than 10% (dispersive) or 30% (non-dispersive) over the mean reference sediment response and statistically significant compared to reference ($\alpha = 0.5$) sediment is considered a hit

BIOACCUMULATION PERFORMANCE CRITERIA BASED ON TISSUE COMPARISONS

ITM performance guidance:

- Tissue concentrations of contaminants are not statistically less than the FDA levels. Therefore, the dredged material is predicted to result in benthic bioaccumulation of contaminants.
- Tissue concentrations of all contaminants are statistically less than FDA levels or there are no levels for the contaminants. In this case, the information is insufficient to reach a conclusion with respect to benthic bioaccumulation of contaminants. The dredged material needs to be further evaluated in Tier III as described in the subsequent bullets.
- Tissue contaminant concentrations following exposure to dredged material which are statistically less than FDA levels, or for which there are no such levels, are compared to tissue contaminant concentrations for organisms similarly exposed to reference sediment:
 - Tissue concentrations of contaminants of concern in organisms exposed to dredged material do not statistically exceed those of organisms exposed to the reference sediment; therefore, the dredged material is predicted not to result in benthic

bioaccumulation of contaminants. However, benthic toxicity effects also have to be considered.

- Tissue concentrations of contaminants of concern in organisms exposed to dredged material statistically exceed those of organisms exposed to reference material. In this case, the conclusion regarding benthic bioaccumulation of contaminants would be based upon technical evaluations that emphasize the various factors deemed appropriate in a particular region. Additional Tier IV may be required.
- Tissue concentrations are above FDA limits but are not statistically different from the reference (or disposal) site. This situation represents an exceptional case, which can only be dealt with at the regional level.

Interpretive guidance for the bioaccumulation test based on the Dredged Material Evaluation and Disposal Procedures (Users Manual) (July 2008):

- Numerical test interpretation guideline or target tissue levels (TTLs) were derived based on human health considerations. The TTLs are allowable tissue concentrations for the bioaccumulation contaminants of concern that were either derived from human-health risk assessments or from FDA action levels. The TTL for mercury is the FDA action level of 1.0 mg/kg wet weight. Interpretation of bioaccumulation results using the one-tailed one-sample t-test (alpha level = 0.05). For undetected chemicals, a concentration equal to one-half the detection limit is used.
 - If the mean tissue concentration of the contaminant of concern is greater than or equal to the TTL, then statistical testing is not required. The conclusion is that the DMMU is not acceptable for aquatic disposal.
 - If the mean tissue concentration of the contaminant of concern is less than the TTL, then a one-tailed, one-sample t-test is conducted and the DMMU is acceptable if the results are not statistically significant.

For an assessment of ecological effects, the results of the test sediment bioaccumulation responses will be compared with the bioaccumulation responses of the reference sediment. Significant bioaccumulation of chemicals of concern in test species relative to reference areas may demonstrate a potential for food-web effects.

- If the results of a statistical comparison show that the tissue concentration of the chemical of concern in test sediment is statistically higher (one-tailed, one-sample, t-test alpha level = 0.1) than the reference sediment, the DMMU will need to be evaluated further to determine the potential ecological significance of the measure tissue resides.

In addition to the performance criteria provided in both the ITM and the PSEP, ADEC requested that the bioaccumulation data be reviewed using an Alaska specific tissue concentration of total mercury of **0.32 ppm wet weight**. This value was chosen based on region-specific information

(State of Alaska Division of Public Health, 2007) and the fish consumption practices for Alaskans. The bioaccumulation data was reviewed and compared using this project specific total mercury value for tissues. The bioaccumulation data was also compared to an ecological risk related value for body burden and documented biological effects (ERED, USACE-ERDC).

3 RESULTS

3.1 FIELD SAMPLING RESULTS

Field sampling was conducted from November 17 to 21, 2008. Sediment was collected from eighteen stations within Douglas Harbor and from five different reference locations within Gastineau Channel. Table 3-1 summarizes the station location information for the Douglas Harbor samples and Figure 3-1 shows the locations on a geo-referenced map. One station, NF08-22, was estimated because coordinates were incorrectly transcribed on field logs. This station was occupied in the correct location based on visual references. The disposal site was also sampled at seven different locations to determine the overall percent fine composition of the sediment. Meg Pinza and Jay Word from NewFields and Andrew Schicht from PND conducted the field sampling. Different participants observed aspects of the field sampling including: John Stone from CBJ, Chris Meade from EPA, Brett Walters USACE and Richard Heffern from ADEC (Figure 3-2).

Table 3-1. Field Sampling Location and Collection Information

Date	Station	Composite	Latitude	Longitude	MLLW Water Depth	Number of Cores	Core Length (ft)
11/17/08	PND07-01	1	58° 16.513	134° 23.131	-6	1	10.5
11/21/08	PND07-02	1	58° 16.478	134° 23.138	+8	3	1.5/1.5/1.5
11/21/08	PND07-03	1	58° 16.494	134° 23.143	+8	3	1.5/1.5/1.5
11/18/08	PND07-04	1	58° 16.473	134° 23.182	-10	1	3.0
11/18/08	PND07-05	2	58° 16.497	134° 23.230	-9	2	4.5/3.1
11/18/08	PND07-06	2	58° 16.506	134° 23.248	-9	2	4.2/3.0
11/19/08	PND07-07	2	58° 16.489	134° 23.223	-8.5	2	2.6/1.5
11/18/08	NF08-17	2	58° 16.496	134° 23.238	-9	2	4.0/5.0
11/21/08	PND07-14	4A	58° 16.527	134° 23.185	-10	1	1.0
11/18/08	PND07-16	4A	58° 16.515	134° 23.163	-11	1	2.5
11/19/08	NF08-19	4A	58° 16.533	134° 23.221	-10.5	1	4.6
11/18/08	NF08-20	4A	58° 16.517	134° 23.189	-10.5	1	7.5
11/19/08	NF08-23	4A	58° 16.504	134° 23.151	-9	1	6.0
11/19/08	PND07-13	4B	58° 16.507	134° 23.232	-11.5	1	4.0
11/18/08	PND07-15	4B	58° 16.501	134° 23.181	-11	1	4.2
11/19/08	NF08-18	4B	58° 16.514	134° 23.237	-7.5	1	5.2
11/19/08	NF-08-21	4B	58° 16.500	134° 23.207	-9	1	5.2
11/19/08	NF08-22	4B	*58° 16.485	134° 23.175	-9.5	1	4.2

* Estimated location based on visual landmarks.



Figure 3-1. Geo-referenced Locations of Sampling Stations within Douglas Harbor



Figure 3-2. Field Group Participants

The sediment samples were kept on blue ice in coolers while in transit to the laboratory in Port Gamble Washington. The sediment was received at the Port Gamble laboratory on November 28, 2008. Contents of the coolers were checked against the chain of custody form, the temperatures inside the coolers were measured upon arrival and ranged between 1 and 6°C; subsequently all samples were transferred to a cold room maintained at $4\pm 2^{\circ}\text{C}$.

3.2 SEDIMENT CORE PROCESSING

The individual cores from each field station were processed on November 30 and December 1. The sediment cores were slit vertically, the core liner spread, and the sediment was inspected. Information regarding sediment type, odor, and color were recorded on the Field Coring Logs.

During core processing distinct vertical layers of differing sediment types were noted and a decision was made to separate the upper and lower segments for each test Dredged Material Management Unit (DMMU 1, 2, 4A and 4B); Figure 3-3 shows an example of the vertical layer(s) observed. The upper layer representing up to approximately 3 feet of sediment was dark black silty organic sediment and the lower part of each core was compact grey sand with a lower percent moisture content compared to the upper sediment layer. The grey sandy sediment was also lower in total organic carbon, which posed a concern for the survival of test organisms in the longer duration bioaccumulation tests. After the sediment from each location was inspected, the sediment from each station and vertical layer was individually mixed to a homogeneous consistency and then an individual 16 oz. archive of sediment was frozen for possible future analysis. Afterwards, the sediment from each field station was combined into testing composites based on the compositing strategy described in the Douglas Harbor SAP.

Whenever two different sediment types were present in one sediment core and the upper material is softer and more pliable, it can coat the core liner from bottom to top with a slick material as the core is pushed into the sediment. This was observed in the cores from Douglas Harbor so care was taken to remove the outer sediment surface that was exposed to the core liner prior to adding sediment to the testing composites.



**Figure 3-3. A) Vertical Layers within the Sediment Core – arrow indicates location of layers
B) Removal of outer sediment surface**

The sediment from the upper sections of each station were combined into an upper test composite and the sediment from the lower section of each core were combined into a lower test composite for each of the DMMUs: 1, 2, 4A and 4B. There were eight test composites for physical and chemical analyses. Figure 3-4 shows the difference in sediment type between the upper and lower composite for Area 4B Comp.



Figure 3-4. Area 4B Upper Comp (left) and Area 4B Lower Comp (right) in Bowls

The results of the mercury analysis showed consistent and comparable concentrations in the upper and lower sediment layers. Section 3.5.2 summarizes the results of the mercury analyses. Preliminary data provided 48 hours after submittal of the sediment samples to the chemistry lab, showed that all of the mercury concentrations in the reference stations and the reference composite were below the project screening level of 0.41 mg/kg. Three test composites (Area 1 Upper Comp, Area 1 Lower Comp, and Area 2 Lower Comp) were detected below the Puget Sound Dredged Disposal Analysis Users Manual (PSDDA) maximum level of 2.1 mg/kg. The remaining test composites were above the PSDDA maximum mercury level. The sediment composition was essentially the same for each of the lower composites; grey compact sand. Therefore, it was considered an appropriate option, to allow for ample sediment for the biological testing, to combine

the material from all of the lower test composites into one testing composite (Lower Comp) for the suite of biological testing.

A variety of aquatic organisms were noted in the sediment cores including the organisms used for the bioaccumulation potential testing; the worm *Nephtys caecoides* and the clam *Macoma nasuta*. Other organisms observed in the sediment cores included a sea urchin *Strongylocentrotus drobachiensis*, several hemichordates (worm-shaped) deutrostomes or acorn worm, and mussels that were present in abundance at the sediment surface layer for many of the stations.

3.3 DISPOSAL SITE CHARACTERIZATION

On November 19, Meg Pinza, Jay Word, Andrew Schicht, Brett Walter, and Peter Wright (captain of the R/V Summer King), collected grab samples from various locations in and around the Gastineau channel. A field estimate of percent fines was determined for each location using sediment to water volume displacement method. The location of each sampling point, the water depth, and percent fines estimate are included in Table 3-2 and locations are shown on Figure 3-5.

Table 3-2. Disposal Site Sample Locations and Characteristics

Date	Station	Description	Latitude	Longitude	Depth (ft)	% Fines
11/19/2008	1	Disposal Site Corner A	58°16.7379	134°23.0205	128	65
11/19/2008	2	Outside of Disposal Site	58°16.412	134°22.408	123	82
11/19/2008	3	Within Disposal Site	58°16.706	134°22.895	128	70
11/19/2008	4	Disposal Site Corner B	58°16.6848	134°22.7908	129	80
11/19/2008	5	Disposal Site Corner C	58°16.7141	134°22.9878	125	50
11/19/2008	6	Disposal Site Corner D	58°16.6219	134°22.8145	126	79
11/19/2008	7	Middle of Disposal Site	58°16.7090	134°22.8634	126	73

Figure 3-6 and Figure 3-7 show the collection of the grab samples, the process of determining the percent fines in the field and the type of sediment present at the disposal site. Sediment consisted mainly of grey brown silt with some cobbles present. *Macoma nasuta* clam shells were present in one of the grab samples collected from the disposal site.

The sediment around the disposal site had a composition of fines that ranged from 50 to 80%. Based on this data set, the decision was made by NewFields, PND, and the regulatory agencies to consider the disposal site heterogeneous in nature. The heterogeneous nature of the disposal site determined the approach to use for collection of the reference sediment. According to the ITM (USACE 1998), if the disposal site is heterogeneous the reference approach can be used to collect reference sediment from a variety of locations and composite the material into one reference composite.

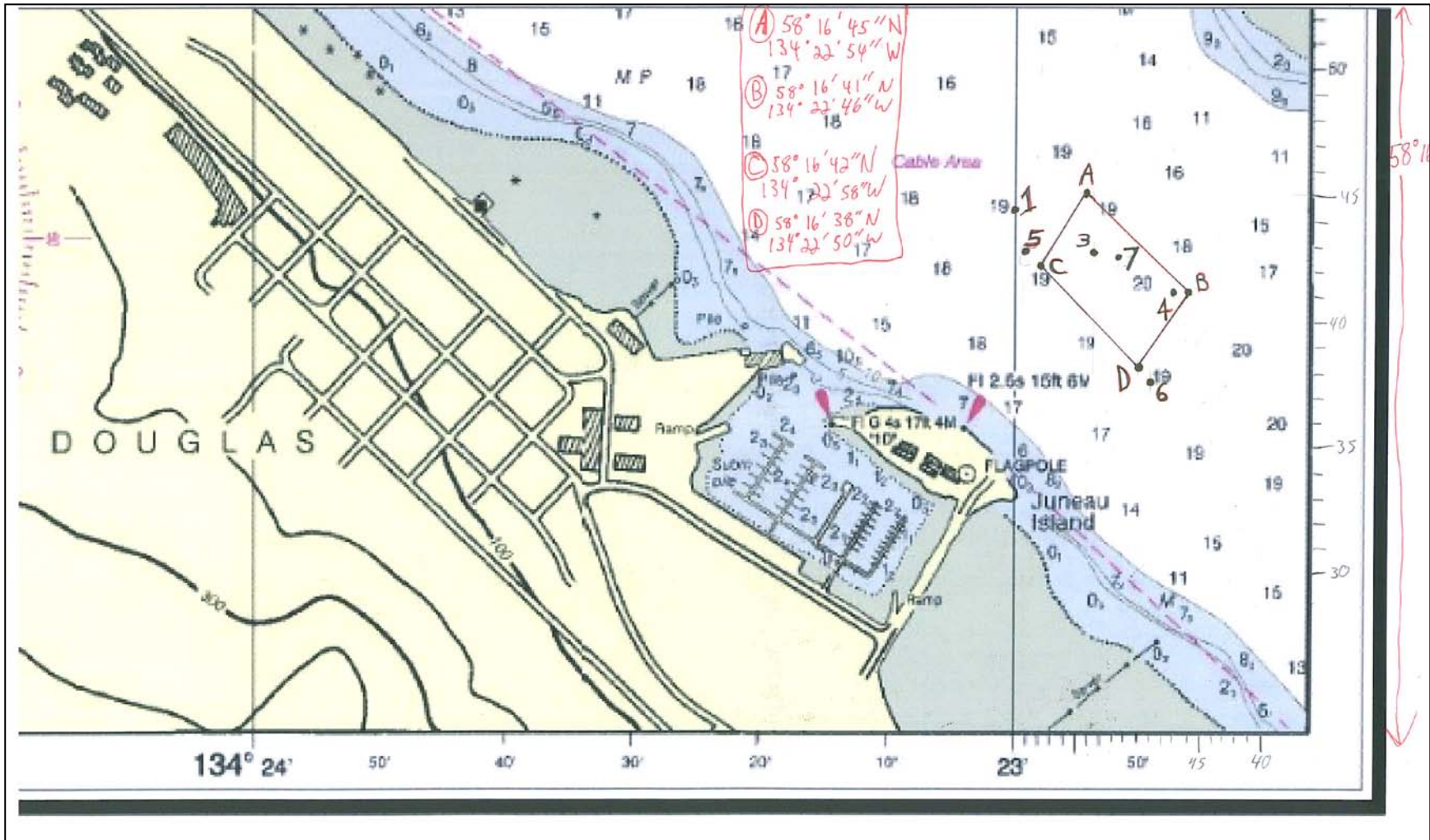


Figure 3-5. Disposal Site Sampling Locations



Figure 3-6 Brett Walters (USACE) and Jay Word (NewFields) collecting grab sample



Figure 3-7. Sediment inside of grab, sieving process, and volume displacement method

3.4 REFERENCE SEDIMENT COLLECTION

On November 20, 2008 Jay Word, Andrew Schicht, and Peter Wright (captain of R/V Summer King), collected reference sediment from five locations in Gastineau Channel. The locations of the reference sites were chosen jointly by NewFields, PND, USACE, EPA, and ADEC at a meeting held on the evening of November 19. The reference locations were chosen to be similar in nature to the disposal site with respect to sediment composition, water depth, total organic carbon, and expected infaunal community. These selected reference sites were also chosen based on historical metals data collected by Rudis, 1996. Locations are shown in Table 3-3 and Figure 3-8.

Table 3-3. Reference Site Locations and Characteristics

Date	Station	Latitude	Longitude	Depth (ft)	% Fines
11/20/2008	REF-01	58°13.192	134°16.224	108	62
11/20/2008	REF-02	58°13.526	134°16.548	103	67
11/20/2008	REF-03	58°13.931	134°17.344	110	55
11/20/2008	REF-04	58°14.330	134°18.055	121	70
11/20/2008	REF-05	58°14.685	134°19.002	120	80

Approximately 10 gallons of sediment were collected from each location using a van Veen grab. Sediment was collected into sediment field bags and stored on blue ice in coolers. The reference sediment was held on blue ice until arrival at the laboratory in Port Gamble Washington. The samples were processed by mixing the sediment from each reference location to a homogeneous consistency. Sediment was collected for chemical analysis from each individual reference site. After each of the five reference sites were prepared in the manner described, a reference composite was created by taking two gallons of sediment from each of the five reference sites. Allocations of sediment were taken from the reference composite and submitted for chemical analysis.

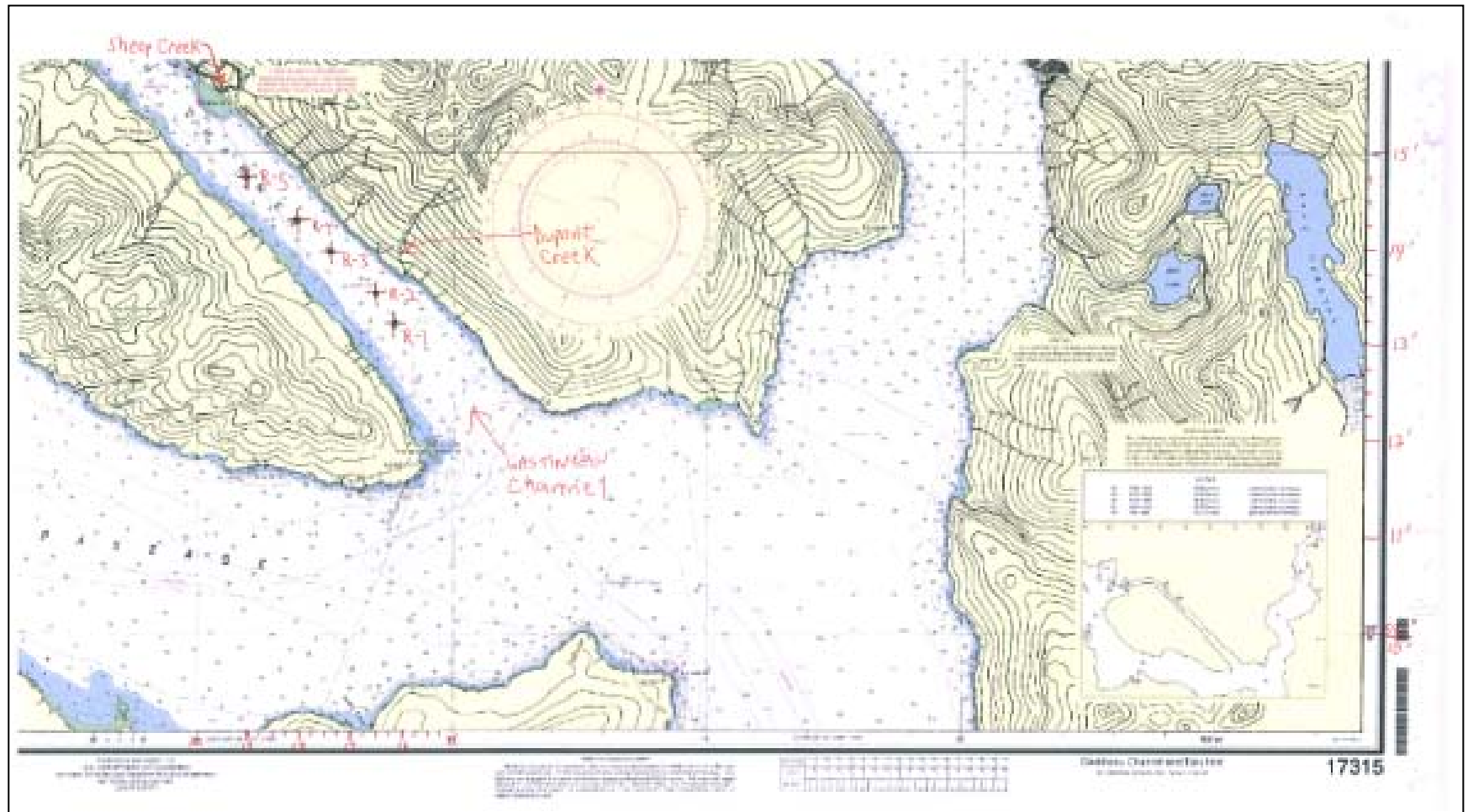


Figure 3-8. Location of Five Reference Site Samples

The consistency of the sediment from the reference areas was similar for the sites, a silty sand environment with cobble present at all locations except for REF-05. The cobble varied among sites with a range of 3 to 8 inches in diameter. The large cobbles were removed during the mixing process as they can interfere with the test results by creating pockets of anaerobic areas that could impact organism survival. A variety of organisms were noted in the sediment from the reference sites and included: brachiopods, dead coral, jingle shells, *Nephtys* sp. worms, and sipunculids. Figure 3-9 show the process of preparing the reference sediment for testing.



Figure 3-9. Sediment mixed and cobble removed. Brachiopod attached to cobble.

Because of the importance of acquiring sediment that is appropriate for the assessment type (infaunal sediment dwellers) and the lack of information on potential locations throughout Gastineau Channel, the Reference Envelope Approach was also recommended. In this case, multiple locations were sampled and handled as separate replicates for the area. This provides a comparison that would address the potential effects in an area rather than at a pre-selected point and allow separately handling the data that is obtained so that multiple sites could be examined. Ideally, all of the sediment would behave a similar manner but if there is an outlier response, that area-replicate could be removed and the data reanalyzed using an unbalanced number of replicates. Reference envelopes are being used in a variety of regions to characterize disposal site environments on a research basis (Puget Sound, San Francisco, and Columbia River). This approach combined with the Reference Area approach that produced a single composite sample from these reference locations was also performed.

3.5 PHYSICAL AND CHEMICAL ANALYSIS OF SEDIMENT

Physical-chemical analyses of the sediment included grain size, total organic carbon (TOC), total solids, and methyl and total mercury were made on subsamples collected from each composite and each station. The results of the physical and chemical characterizations are summarized in the following sections. The laboratory bench sheets for the chemical and physical analyses of sediments are presented in Appendix B.

All samples were received and analyzed within the acceptable holding times. A discussion of the QA/QC data for each analyte is presented in the following sections and in the QA/QC Summaries in Appendix B.

3.5.1 SEDIMENT GRAIN SIZE, TOTAL ORGANIC CARBON, AND TOTAL SOLIDS

Grain size results for each of the test and reference composites and individual reference stations are presented in Table 3-4. The Douglas Harbor test sediment cores were split into two test composites based on apparent grain-size with the finer silt and clay material in the upper composites and the silt and sand material in the lower composites. The grain size analysis confirmed the difference in grain size between the upper and lower layers. The upper composites with the exception of Area 1 Upper Comp were greater than 86% silt and clay while the lower composites samples were greater than 90% sand and silt. The location for Area 1 was located on the north side of the harbor and was exposed at low tide when samples were collected. The higher sand content is not unexpected given its location.

The percentage of silt and clay from the reference sites ranged from 54.7% at REF-02 to 94.4% at REF-05 encompassing the range of grain sizes observed for the Douglas Harbor test composites.

The total organic carbon content was also different between the top and bottom layers of each composite with the TOC levels in the bottom composites ranging from 0.047 to 0.069 and 0.621 to 1.88 in the upper composites. The highest TOC was associated with Area 1 Upper Comp which again may be related to the fact that it was an exposed site at low tide, with marine plant growth present on the sediment surface.

Total organic carbon in the individual reference stations and the reference composite were similar to the upper test composites with values ranging from 0.544 to 0.919. Percent solids were calculated for all of the test and reference composites with a pattern of more water in the upper composite sediment (61.0 to 64.3% solids) than in the lower composite sediments (80.8 to 84% solids). Again the individual reference samples and reference composite most closely aligned with the upper composites with total solids ranging from 50.4 to 64.9%.

ARI conducted the physical analysis of the sediment and included a matrix spike, a duplicate, a laboratory control sample, a method blank, and a standard reference material with the batch of TOC samples. The individual sample from REF-03 was used for the matrix spike and the duplicate analysis and all of the quality assurance data are provided in Appendix B. The matrix spike percent recovery was 121.3%, the relative percent difference for total organic carbon and total solids were 3.5% and 2.6% respectively, the laboratory control sample percent recovery was 101.6%, the blanks had undetected concentrations of total organic carbon, and the percent recovery of the total organic carbon standard reference material (SRM) was 92.5%. All of these measures are within the quality criteria established by the method indicating the data are usable for interpretation.

Table 3-4. Summary of Conventional Information, Douglas Harbor

Sample ID	% Gravel	% Sand	Silt (%)	Clay (%)	TOC (%)	% Solids
Area 1 Upper Comp	14.5	46.0	27.4	12.0	1.88	64.3
Area 1 Lower Comp	0.4	41.6	50.8	7.3	0.067	84.0
Area 2 Upper Comp	0.7	14.6	63.4	21.3	0.621	65.8
Area 2 Lower Comp	0.0	47.4	49.5	2.9	0.047	81.0
Area 4A Upper Comp	2.9	11.6	60.5	25.2	0.798	61.1
Area 4A Lower Comp	0.2	34.3	56.2	9.2	0.069	81.8
Area 4B Upper Comp	2.4	10.9	65.1	21.7	0.837	63.8
Area 4B Lower Comp	0.0	23.3	68.9	7.8	0.055	80.3
REF -01	14.0	26.5	38.5	20.9	0.562	63.0
REF-02	19.3	26.0	34.9	19.8	0.544	64.9
REF-03	6.1	28.3	42.3	23.4	0.687	60.1
REF-03 Lab Dup	NA	NA	NA	NA	0.646	62.7
REF-03 Lab Dup	NA	NA	NA	NA	0.647	63.1
REF-04	19.4	14.8	40.3	25.4	0.735	52.9
REF-05	0.1	5.6	61.0	33.4	0.919	50.4
REF-05 Lab Dup	0.3	5.8	60.5	33.4	NA	NA
REF-05 Lab Dup	0.0	6.2	60.2	33.6	NA	NA
REF-Comp	8.3	20.5	45.7	25.4	0.706	60.0

3.5.2 METHYL MERCURY AND TOTAL MERCURY IN SEDIMENT

Methyl and total mercury were analyzed in the test composites, individual reference samples and the reference composite (Table 3-5). Methyl mercury concentrations are reported in ng/g and represent the organic form of mercury that is more easily absorbed into the living tissue of aquatic organisms, is not easily eliminated, accumulates in organisms and may be transferred up the food chain. The degree to which mercury is transformed into methyl mercury and transferred up the food chain through bioaccumulation depends on factors such as water chemistry and the complexity of the food web.

The concentration of methyl mercury in the sediment ranged from 0.796 ng/g in sediment from Area 2 Lower Comp to 3.46 ng/g in sediment from Area 4A Lower Comp. The methyl mercury concentration in the individual reference samples and reference composite were lower, ranging from 0.277 in the REF Comp to 0.350 for REF-05.

Concentrations of total mercury measured in the composites were similar between the upper and lower layers and ranged from 1.11 µg/g to 3.22 µg/g. This range of total mercury concentrations were similar to those reported in the sediment samples collected in 2007 that ranged from 1.7 to 3.5 µg/g (Data taken from PND Report #062065, p. 10). The concentration of total mercury in the reference samples and reference composite were lower than those found in Douglas Harbor and ranged from 0.178 to 0.303 µg/g.

QA/QC measures were within quality control limits established in Table 2-4 for the blanks, standard reference materials, the matrix spikes and the replicate analysis, a summary of the quality assurance data is provided in Appendix B.

Table 3-5. Methyl and Total Mercury in Sediment, Douglas Harbor

Sample ID	% Dry Weight	Methyl Mercury (ng/g dry weight)	Total Mercury (µg/g dry weight)
Area 1 Upper Comp	61.8	2.47	1.11
Area 1 Lower Comp	82.9	3.05	1.29
Area 2 Upper Comp	60.1	0.802	2.50
Area 2 Lower Comp	80.7	0.796	1.97
Area 4A Upper Comp	61.6	1.34	3.22
Area 4A Lower Comp	80.8	3.46	2.21
Area 4A Lower Comp	80.8	3.33	2.56
Area 4B Upper Comp	64.9	1.08	2.33
Area 4B Lower Comp	80.9	2.44	3.18
REF -01	64.5	0.294	0.178
REF-02	63.2	0.308	0.195
REF-03	63.3	0.314	0.199
REF-04	55.9	0.445	0.268
REF-05	51.9	0.350	0.303
REF-Comp	58.7	0.277	0.226

3.5.3 AVS AND SEM METALS

Acid volatile sulfides (AVS) and simultaneously extracted metals data are used to determine whether sulfides are an important factor controlling the biological availability of metals in test sediments. The AVS in sediments bind to certain metals such that sediment-dwelling organisms are not likely to be exposed to the toxic potential of these metals. The SEM/AVS ratio is used to estimate if metals present in sediments are available for uptake into the tissues of aquatic organisms. If there is more AVS in sediments than metals, then the metals present in the sediments are not likely to cause adverse effects in the aquatic community near these sediments.

Each test composite, individual reference sample and the reference composite were analyzed for AVS and SEM metals. Data for the individual reference samples and the composites are presented in Table 3-6. For the Douglas Harbor composites, only one composite, Area 1 Lower Comp had a SEM/AVS ratio greater than one, indicating that for this composite the AVS is not sufficient to bind all of the SEM metals. For this composite the SEM metals could be available to sediment dwelling organisms. However, based on the solubility products for metals, mercury is the first to bind to AVS followed by Cu, Cd, Pb, Ni, and then Zn (Casas and Crecelius 1994). This means that the mercury is bound with the AVS and not available for aquatic organism uptake. The SEM/AVS

ratio was also greater than one for REF-01 through REF-04. However, given the very low concentrations of mercury in the reference samples, mercury was not expected to accumulate in tissues of organisms exposed to the reference site samples.

A QA/QC Summary is provided in Appendix B. QA/QC measures were all within target ranges, with a few exceptions. Trace amounts of SEM metals were detected in the blanks at concentrations below the sample concentrations. Sample concentrations that were less than three times the method detection limit are flagged with a J and should be considered estimates. One replicate pair for AVS had a calculated RPD greater than 25%. The RPD for SEM ranged from 0 to 35%. One replicate pair for cadmium had a RPD of 27% and one replicate for copper had an RPD of 35%. All other QC data, blank spikes, matrix spikes, and, SRM were within the data quality criteria set for the method.

Table 3-6. Concentrations of AVS and SEM Metals in Sediment (µmoles/g DW)

Sample ID	Dry Weight (%)	AVS (µmoles/g DW)	SEM/AVS Ratio	Cd	Cu	Hg	Ni	Pb	Zn
				SEM (µmoles/g DW)					
Area 1 Upper Comp	61.2	35.6	0.0391	0.00316	0.353	0.000167	0.137	0.131	0.765
Area 1 Lower Comp	82.2	0.319	1.12	0.000529	0.0745	0.000681	0.0393	0.0374	0.206
Area 2 Upper Comp	64.3	56.4	0.0299	0.00746	0.291	0.000401	0.156	0.133	1.10
Area 2 Lower Comp	81.0	0.701	0.479	0.000727	0.0450	0.000459	0.0372	0.0313	0.221
Area 4A Upper Comp	61.8	61.8	0.0261	0.00355	0.375	0.000194	0.147	0.162	0.923
Area 4A Upper Comp	61.8	71.7	0.0201	0.00315	0.262	0.000175	0.144	0.159	0.875
Area 4 A Lower Comp	80.9	6.50	0.0656	0.000888	0.0677	0.000591	0.0367	0.0583	0.262
Area 4B Upper Comp	61.6	69.9	0.0187	0.00298	0.243	0.0000982	0.135	0.148	0.779
Area 4B Lower Comp	80.6	4.98	0.0854	0.000680	0.0750	0.00112	0.0296	0.0882	0.231
REF -01	66.6	0.444	1.76	0.000640	0.161	0.0000643	0.111	0.0786	0.429
REF -01	64.0	0.578	1.44	0.000843	0.177	0.0000661	0.111	0.0847	0.458
REF-02	65.2	0.505	1.81	0.000731	0.191	0.0000783	0.126	0.0903	0.505
REF-03	63.4	0.506	1.69	0.000811	0.182	0.0000495	0.116	0.0924	0.462
REF-04	55.1	0.258 J	4.97	0.000826	0.447	0.000109	0.132	0.131	0.573
REF-05	52.3	6.00	0.236	0.00110	0.272	0.0000391	0.159	0.166	0.817
REF-Comp	60.2	1.62	0.611	0.000565	0.206	0.0000793	0.127	0.109	0.545

3.5.4 METHYL MERCURY AND TOTAL MERCURY IN PORE WATER

Methyl mercury and total mercury were analyzed in the pore water associated with each of the test and reference composites and the individual reference site samples (Table 3-7). Three composite samples from the lower layer were sufficiently dry that they did not produce pore water; therefore no measurements could be made for these samples. QA/QC measures were within quality control limits established in Table 2-4 for the blanks, standard reference materials, matrix spikes and the replicate analysis; a summary of the quality assurance data is provided in Appendix B.

The concentration of methyl mercury in the pore water samples from the Douglas Harbor test composites ranged from 0.225 ng/L (Area 2 Upper and Area 4B Upper) to 0.979 ng/L in 4A Lower Comp. The methyl mercury concentration in the individual reference samples and reference composite were 0.393 ng/L in the REF-Comp to 1.90 ng/L in REF-04. Total mercury concentrations ranged from 13.1 ng/L to 29.2 ng/L in Douglas Harbor composite samples and from 4.11 ng/L to 19.4 ng/L in the Reference area samples.

Table 3-7. Concentrations of Methyl and Total Mercury in Water

Sample ID	Methyl Mercury (ng/L)	Total Mercury (ng/L)
Area 1 Upper Comp	0.347	13.1
Area 1 Lower Comp	NM	NM
Area 2 Upper Comp	0.225	25.3
Area 2 Lower Comp	NM	NM
Area 4A Upper Comp	0.382	14.8
Area 4A Lower Comp	0.979	29.2
Area 4B Upper Comp	0.225	17.4
REF-01	0.405	5.10
REF-02	1.36	10.3
REF-03	0.582	10.7
REF-04	1.90	19.4
REF-05	0.147	4.11
REF-Comp	0.433	8.83
REF-Comp Dup	0.393	8.09

3.6 BENTHIC TEST RESULTS

This section presents a summary of the benthic tests conducted in support of Douglas Harbor project. All of the results and bench sheets for this test are provided in Appendix C. Ammonia and sulfide data were collected from the bulk pore water to determine if acclimation of test sediment was required; the bulk pore water measurements are summarized in Table 3-8; no pore water could be extracted from the Lower Comp. Area 4B Upper was selected for acclimation based on ammonia concentrations in the bulk pore water and the Lower Comp sample was acclimated due to the deep burial of the sediment and the potential isolation from biogenic processes. Testing of acclimated sediment in addition to the normal testing provides a measurement of the contribution of these factors to any observed toxicity.

In addition to the REF-COMP sample, the five individual reference samples were also tested; the mean of these results is referred to as REF-X in the following sections.

Table 3-8. Summary of Water Quality in the Bulk Pore Water

Sample ID	Total Ammonia (mg/L)	Total Sulfides (mg/L)	pH	Salinity (ppt)
Area 1 Upper	15.8	0.2	7.1	25
Area 2 Upper	15.9	0.486	7.7	21
Area 4A Upper	23.1	0.29	7.6	27
Area 4B Upper	36.6	0.502	7.7	27
REF-01	2.18	0.155	7.3	32
REF-02	3.4	0.267	7.3	32
REF-03	4.28	0.498	7.2	32
REF-04	4.43	0.125	7.2	32
REF-05	3.87	0.077	7.2	32
REF-Comp	2.57	0.125	7.2	32

3.6.1 RESULTS OF BENTHIC TEST WITH *AMPELISCA ABDITA*

The 10-day amphipod test with *A. abdita* was initiated on January 9, 2009 and was validated by 91% survival in the control treatment (Table 3-9). Measurements of DO, pH, salinity, and temperature were within recommended limits throughout the test (Tables 3-10 and 3-11).

The LC₅₀ for the cadmium reference-toxicant test was calculated at 0.74 mg Cd/L, this value is within the control chart limits (0.14 to 1.1 mg Cd/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory. The LC₅₀ for the ammonia reference-toxicant test was 24.7 mg/L. Ammonia values in the test treatments were all less than the LC₅₀ except for Area 4B which had an initial pore water ammonia concentration of 45.9 mg/L. According to the Puget Sound Dredged Material Evaluation and Disposal Procedures (USACE 2008), total ammonia values greater than 30 mg/L in the pore water is considered a threshold value that could require the sediment to be purged prior to testing. Instead of purging the sediment, Area 4B Comp was acclimated because of the potential high ammonia in the pore water interfering with the outcome of the test. The initial pore water ammonia value for the acclimated treatment was 6.22 mg/L. Survival for Area 4B was 87% and survival for Area 4B acclimated was 94%.

Mean survivals in the reference treatments were 93% in REF-Comp, 90% in REF-Comp acclimated, 95% in REF-X, and 96% in REF-X acclimated. Mean percentage survival in the test composites ranged from 76% to 94%. The survival data for *A. abdita* were arcsine-square root transformed prior to statistical comparison. The transformed data exhibited a normal distribution and equal distribution, therefore the statistical comparison was performed with ANOVA and LSD (see Section 2.7 for discussion of statistical methods).

Only the Lower Comp sample was significantly lower in survival ($p \leq 0.05$) than survival in the REF-Comp sediment. Survival of amphipods in the acclimated Lower Comp sediment was 94% and not statistically different than the REF-Comp survival.

Table 3-9. Survival Summary for the 10-Day Benthic Test with *Ampelisca abdita*.

Sample ID	Mean survival (%)	Standard Deviation	Significantly Less Than REF-Comp?
Control	91	4.2	--
REF-Comp	93	2.7	--
REF-Comp Acclimated	90	7.1	--
REF-X	95	6.1	--
REF-X Acclimated	96	4.2	--
Area 1 Comp	92	6.7	No
Area 2 Comp	92	5.7	No
Area 4A Comp	90	10.0	No
Area 4B Comp	87	5.7	No
Area 4B Comp Acclimated	94	6.5	No
Lower Comp	76	11.4	Yes
Lower Comp Acclimated	94	5.5	No

Table 3-10. Water Quality Summary for the 10-Day Benthic Test with *Ampelisca abdita*.

Sample ID	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	7.8	7.4	8.1	19.4	19.0	19.8	8.0	7.7	8.3	29.4	29.0	31.0
Area 1 Comp	7.8	6.8	8.0	19.4	19.0	19.8	8.1	7.9	8.3	29.5	29.0	31.0
Area 2 Comp	7.8	7.2	8.0	19.5	19.0	19.9	8.1	7.9	8.4	29.6	29.0	31.0
Area 4A Comp	7.8	7.4	8.1	19.4	19.0	19.8	8.0	7.7	8.3	29.4	29.0	31.0
Area 4B Comp	7.8	6.8	8.0	19.4	19.0	19.8	8.1	7.9	8.3	29.5	29.0	31.0
Area 4B acclimated	7.8	7.2	8.0	19.5	19.0	19.9	8.1	7.9	8.4	29.6	29.0	31.0
REF Comp	7.8	7.4	8.1	19.4	19.0	19.8	8.0	7.7	8.3	29.4	29.0	31.0
REF Comp acclimated	7.8	6.8	8.0	19.4	19.0	19.8	8.1	7.9	8.3	29.5	29.0	31.0
Lower Comp.	7.8	7.3	8.1	19.4	18.9	19.9	8.0	7.7	8.2	29.3	29.0	31.0
Lower Comp acclimated	7.7	6.5	8.0	19.4	19.0	19.0	8.0	7.8	8.1	31.0	30.0	33.0
REF-X	7.8	7.3	8.0	19.5	19.2	19.2	8.0	7.9	8.2	29.6	29.0	31.0
REF-X - acclimated	7.8	6.8	8.1	19.4	19.0	19.0	8.0	7.9	8.3	30.6	29.0	34.0

Table 3-11. Test Conditions for *Ampelisca abdita*.

Test Conditions for <i>A. abdita</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	7 week	
Acclimation of test sediment	Approximately 1 week prior test initiation for Area 4B Comp and Lower Comp	
Control sediment	Tomales Bay, California (native sediment)	
Test Species	<i>Ampelisca abdita</i>	
Supplier	John Brezina	
Date acquired	1/6/09	
Organism acclimation/holding time	4 days	
Age class	Adult	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	10-Day static	
Test dates	1/9/09 – 1/19/09	
Control water	0.45 µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 18.8 – 20.1 °C
Test Salinity	Recommended: 30 ± 2 ppt	Achieved: 29 – 31 ppt
Test dissolved oxygen	Recommended: > 4.6 mg/L	Achieved: 5.6 – 7.8 mg/L
Test pH	Recommended: 8.0 ± 0.5	Achieved: 7.8 – 8.8
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 9%
Reference Toxicant LC50	0.74 mg/L Cd	
Acceptable Range	0.14 – 1.1 mg/L	
Test Lighting	Continuous	
Test chamber	1-Liter Glass Chamber	
Replicates/treatment	5 + 2 surrogates for measuring pore water ammonia levels	
Organisms/replicate	20	
Exposure volume	175 mL sediment/ 950 mL water	
Feeding	None	
Water renewal	None	
Deviations from Test Protocol	None	

3.6.2 RESULTS OF BENTHIC TEST WITH *NEANTHES ARENACEODENTATA*

The benthic test with *N. arenaceodentata* was initiated on December 13, 2008 and was validated by 100% survival in the controls (Table 3-12). All water quality parameters fell within the acceptable limits throughout the duration of the 10-day test (Tables 3-13 and 3-14Table 3-13).

The LC₅₀ for the cadmium reference-toxicant test was calculated at 10.2 mg Cd/L, this value is within the control chart limits (2.8 – 16.2 mg Cd/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory. The LC₅₀ for the ammonia reference-toxicant test was 125.5 mg/L. The highest ammonia values measured in the test treatments was 12.1 mg/L in Area 4B Comp. The PSDDA Users Manual (2008) provides a threshold total ammonia value in the pore water of 10 mg/L at which no effects on survival or growth are expected to occur.

Mean survival was 96% in REF-Comp, and 92% for the REF-X samples. Mean percentage survival in the test composites ranged from 84% to 100%. Survival data were arcsine-square root transformed prior to statistical testing. Data were normally distributed, but variances were not equal therefore a one-tailed t-test was performed to compare to the REF-Comp sample. Results of the statistical analysis showed that none of the test composites had survival that was statistically lower than the reference composite.

Table 3-12. Survival Summary for the 10-Day Benthic Test with *Neanthes arenaceodentata*.

Sample ID	Mean survival (%)	Standard Deviation	Significantly Less Than REF-Comp?
Control	100	0.0	--
REF-Comp	96	8.9	--
REF-X	92	11.0	No
Area 1 Comp	96	8.9	No
Area 2 Comp	88	17.9	No
Area 4A Comp	92	11.0	No
Area 4B Comp	100	0.0	No
Lower Comp	84	16.7	No

Table 3-13. Water Quality Summary for the Benthic Test with *Neanthes arenaceodentata*.

Sample ID	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	6.9	6.4	7.7	19.6	19.2	19.9	8.1	7.9	8.3	30.3	29.0	32.0
REF-Comp	6.6	5.0	8.0	19.6	19.0	20.0	8.1	7.7	8.5	29.7	29.0	31.0
REF-X	6.8	6.2	7.7	19.5	19.0	19.8	8.2	7.9	8.7	29.7	29.0	31.0
Area 1 Comp	6.9	6.4	7.7	19.6	19.2	19.9	8.1	7.9	8.3	30.3	29.0	32.0
Area 2 Comp	6.6	5.0	8.0	19.6	19.0	20.0	8.1	7.7	8.5	29.7	29.0	31.0
Area 4A Comp	6.8	6.2	7.7	19.5	19.0	19.8	8.2	7.9	8.7	29.7	29.0	31.0
Area 4B Comp	6.9	6.4	7.7	19.6	19.2	19.9	8.1	7.9	8.3	30.3	29.0	32.0
Lower Comp	6.6	5.0	8.0	19.6	19.0	20.0	8.1	7.7	8.5	29.7	29.0	31.0

Table 3-14. Test Conditions for *Neanthes arenaceodentata*.

Test Conditions: <i>N. arenaceodentata</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	3 week	
Control sediment	Yaquina Bay, Oregon	
Test Species	<i>N. arenaceodentata</i>	
Supplier	Don Reish	
Date acquired	12/13/2008	
Organism acclimation/holding time	0	
Age class	Juvenile	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	10-Day static	
Test dates	12/13/2008-12/23/2008	
Control water	0.45 µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 19.0 – 20.0 °C
Test Salinity	Recommended: 30 ± 2 ppt	Achieved: 29 - 32 ppt
Test dissolved oxygen	Recommended: > 4.6 mg/L	Achieved: 5.0 – 8.0 mg/L
Test pH	Recommended: 8.0 ± 0.5	Achieved: 7.7 – 8.7
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 0%
Reference Toxicant LC50	10.2 mg/L	
Acceptable Range	2.8 – 16.2 mg/L	
Test Lighting	Continuous	
Test chamber	1-Liter Glass Chamber	
Replicates/treatment	5 + 2 surrogates for measuring porewater ammonia levels	
Organisms/replicate	5	
Exposure volume	175 mL sediment/ 950 mL water	
Feeding	None	
Water renewal	None	
Deviations	None	

3.7 WATER-COLUMN TEST RESULTS

The results of the water-column toxicity tests are presented in this section. The water-column tests were performed with the mysid shrimp, *Americamysis bahia*, the fish, *Menidia beryllina*, and the larvae of the bivalve, *Mytilus* sp.

3.7.1 RESULTS OF THE WATER-COLUMN TEST WITH *AMERICAMYSIS BAHIA*

The water-column test with *A. bahia* was initiated on December 17, 2008 and was validated by 98% survival in the seawater and site water controls (Table 3-15). All water quality parameters fell within the acceptable testing limits throughout the duration of the 96-hour test (Tables 3-16 and Table 3-17).

The LC₅₀ for the copper reference-toxicant test was calculated to be 242 µg Cu/L, this value is within the control chart limits (137 - 413 mg Cu/L) for this species, indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory.

The LC₅₀ for the ammonia reference-toxicant test was 70 mg/L. Ammonia values in the test treatments were all less than the LC₅₀, the highest concentration measured in the test treatments was 20.5 mg/L.

Mean percentage survival in the 100% concentration for each of the composites ranged from 98% to 100%, and the estimated LC₅₀ for each of the test treatments was >100%. Statistical comparison of the 100% concentrations of test treatment survival to control survival showed all five test treatments were not statistically lower in survival than the control; further, survival of *A. bahia* in all test concentrations were 98% or greater which is above the test performance criteria for control samples (90%).

Table 3-15. Survival Summary for *Americamysis bahia*.

Sample ID	Concentration (%)	Mean survival (%)	Standard Deviation	Statistically Less Than Control?
Control	0	98	4.5	--
Site Water	0	98	4.5	--
Area 1 Comp	10	96	5.5	--
	50	100	0.0	--
	100	98	4.5	No
Area 2 Comp	10	98	4.5	--
	50	98	4.5	--
	100	98	4.5	No
Area 4A Comp	10	98	4.5	--
	50	100	0.0	--
	100	100	0.0	No
Area 4B Comp	10	100	0.0	--
	50	100	0.0	--
	100	100	0.0	No
Lower Comp	10	98	4.5	--
	50	100	0.0	--
	100	100	0.0	No

Table 3-16. Water Quality Summary for the Water Column Test with *Americamysis bahia*.

Sample ID	Water-Column Conc. (%)	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	0	6.0	5.2	7.6	19.6	19.0	20.3	26.1	25.0	27.0	7.5	7.3	7.8
Site Water	0	6.2	5.3	8.7	19.6	19.2	20.0	24.9	24.0	26.0	7.7	7.4	7.9
Area 1 Comp	10	5.8	4.9	7.7	19.7	19.2	20.3	25.8	25.0	26.0	7.7	7.4	7.9
	50	5.8	4.9	7.5	19.7	19.1	20.5	25.8	25.0	26.0	7.8	7.4	7.9
	100	5.4	4.6	7.0	19.7	19.2	20.2	24.9	24.0	25.0	7.9	7.4	8.0
Area 2 Comp	10	5.7	4.9	7.5	19.8	19.2	20.7	25.8	25.0	26.0	7.7	7.5	8.0
	50	5.8	5.1	7.6	19.8	19.2	20.5	25.7	25.0	26.0	7.8	7.6	8.0
	100	5.6	4.8	7.5	19.8	19.2	21.2	24.9	24.0	25.0	7.9	7.8	8.1
Area 4A Comp	10	5.8	5.2	7.4	19.8	19.2	21.1	25.8	25.0	26.0	7.8	7.7	8.0
	50	5.8	5.2	7.3	19.8	19.2	20.7	25.8	25.0	26.0	7.8	7.7	8.0
	100	5.7	5.3	6.8	19.8	19.4	20.6	24.9	24.0	26.0	8.0	7.8	8.1
Area 4B Comp	10	5.8	5.1	7.4	19.7	19.3	20.4	25.8	25.0	26.0	7.8	7.7	8.0
	50	5.6	4.7	7.5	19.7	19.3	20.7	25.8	25.0	26.0	7.9	7.7	8.0
	100	5.5	4.7	7.4	19.7	19.2	20.5	25.7	25.0	26.0	8.0	7.8	8.1
Lower Comp	10	5.7	5.2	7.4	19.7	19.3	20.4	25.7	25.0	26.0	7.8	7.4	8.0
	50	5.8	5.0	7.7	19.7	19.3	20.3	25.8	25.0	26.0	7.8	7.5	8.0
	100	6.0	5.4	7.8	19.6	19.2	20.1	24.8	24.0	25.0	7.8	7.6	8.0

Table 3-17. Test Conditions for *Americamysis bahia*

Test Conditions: <i>A. bahia</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	4 weeks	
Test Species	<i>Americamysis bahia</i>	
Supplier	Aquatic BioSystems	
Date acquired	12/16/08	
Organism acclimation/holding	1 day	
Age class	4 days old	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	96-hour SPP	
Test dates	12/17/08 – 12/21/08	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 19.0 – 21.2°C
Test Salinity	Recommended: 25 ± 2 ppt	Achieved: 24 - 27 ppt
Test dissolved oxygen	Recommended: > 3.7 mg/L	Achieved: 4.6 – 8.7 mg/L
Test pH	Recommended: 7.8 ± 0.5	Achieved: 7.3 – 8.1
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 2%
Reference Toxicant LC50	242 µg Cu/L	
Acceptable Range	137 - 413 µg Cu/L	
Test Lighting	16- hours light, 8-hours dark	
Test chamber	600mL Glass Chamber	
Replicates/treatment	5	
Organisms/replicate	10	
Exposure volume	250mL	
Feeding	Twice daily	
Water renewal	None	
Deviations from Test Protocol	None	

3.7.2 RESULTS OF THE WATER-COLUMN TEST WITH *MENIDIA BERYLLINA*

The water-column test with *M. beryllina* was initiated on December 16, 2008 and was validated by 100% survival in the seawater control and 98% survival in the site water control (Table 3-18). All water quality parameters fell within the acceptable limits throughout the duration of the 96-hour test (Table 3-19 and Table 3-20).

The LC₅₀ for the copper reference-toxicant test was 307 µg Cu/L, and was inside the control chart limits (90 – 443 µg Cu/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory. The LC₅₀ for the ammonia reference-toxicant test was 62.1 mg/L. Ammonia values in the test treatments were all less than the LC₅₀ with highest measured ammonia concentration of 13.1 mg/L.

Mean percentage survival in the 100% concentration for each of the composite samples ranged from 96% to 100%, and the estimated LC₅₀ for each of the test treatments was >100%.

Table 3-18. Survival Summary for *Menidia beryllina*.

Sample ID	Water-Column Concentration (%)	Mean survival (%)	Standard Deviation	Statistically Less Than Control?
Control	0	100	0.0	--
Site Water	0	98	4.5	--
Area 1 Comp	10	100	0.0	--
	50	100	0.0	--
	100	100	0.0	No
Area 2 Comp	10	100	0.0	--
	50	98	4.5	--
	100	100	0.0	No
Area 4A Comp	10	98	4.5	--
	50	100	0.0	--
	100	100	0.0	No
Area 4B Comp	10	98	4.5	--
	50	96	5.5	--
	100	98	4.5	No
Lower Comp	10	100	0.0	--
	50	100	0.0	--
	100	100	0.0	No

Table 3-19. Water Quality Summary for the Water Column Test with *Menidia beryllina*.

Sample ID	Water-Column Conc. (%)	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control		6.8	6.1	7.3	19.7	18.9	20.3	26.0	25.0	27.0	7.8	7.4	7.9
Site Water		6.5	5.8	7.1	19.6	19.2	20.3	25.1	24.0	26.0	7.8	7.3	8.0
Area 1 comp	10	6.5	6.0	6.7	19.8	19.3	20.6	26.0	25.0	27.0	7.9	7.5	8.0
	50	6.6	6.1	6.9	19.7	19.2	20.8	26.0	25.0	27.0	7.9	7.6	8.1
	100	6.3	5.5	6.7	19.6	19.1	20.2	25.1	24.2	26.0	7.9	7.6	8.1
Area 2 comp	10	6.7	6.3	7.1	19.8	19.4	21.1	25.9	25.0	27.0	7.9	7.6	8.1
	50	6.5	6.1	6.8	19.8	19.5	20.8	25.7	25.0	26.0	8.0	7.7	8.1
	100	6.2	5.2	6.6	19.8	19.3	21.3	25.0	24.0	26.0	8.0	7.7	8.2
Area 4A comp	10	6.5	6.1	6.9	19.8	19.1	21.0	26.2	25.0	27.0	7.9	7.7	8.1
	50	6.5	6.2	6.7	19.7	19.4	20.8	25.9	25.0	27.0	8.0	7.7	8.1
	100	6.4	6.0	6.8	19.6	19.2	20.3	25.7	25.0	27.0	8.1	7.8	8.2
Area 4B comp	10	6.4	6.2	6.7	19.7	19.2	20.3	26.0	25.0	27.0	7.9	7.6	8.0
	50	6.2	5.8	6.6	19.7	19.3	20.5	25.7	25.0	26.0	8.1	7.7	8.2
	100	6.0	4.7	6.4	19.6	19.3	20.5	25.7	25.0	26.0	8.2	7.8	8.3
Lower Comp	10	6.3	5.8	6.9	19.6	19.2	20.5	25.7	25.0	26.0	7.9	7.7	8.1
	50	6.4	6.0	6.8	19.6	19.2	21.1	25.4	25.0	26.0	7.9	7.7	8.0
	100	6.5	6.2	6.7	19.6	19.1	20.8	25.1	24.0	26.0	7.9	7.6	8.1

Table 3-20. Test Conditions for *Menidia beryllina*.

Test Conditions: <i>M. beryllina</i>		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	4 weeks	
Test Species	<i>M. beryllina</i>	
Supplier	Aquatic BioSystems	
Date acquired	12/13/08	
Organism acclimation/holding time	3 days	
Age class	10 days old	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	96-hour SPP	
Test dates	12/16/08 – 12/20/08	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 20 ± 1 °C	Achieved: 18.9 – 21.3°C
Test Salinity	Recommended: 25 ± 2 ppt	Achieved: 24- 27 ppt
Test dissolved oxygen	Recommended: > 3.7 mg/L	Achieved: 4.7- 7.3 mg/L
Test pH	Recommended: 7.8 ± 0.5	Achieved: 7.3 - 8.2
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 0%
Reference Toxicant LC50	307 µg Cu/L	
Acceptable Range	90- 443 µg Cu/L	
Test Lighting	16- hours light, 8-hours dark	
Test chamber	600mL Glass Chamber	
Replicates/treatment	5	
Organisms/replicate	10	
Exposure volume	250 mL	
Feeding	Once at 48 hours	
Water renewal	None	
Deviations	None	

3.7.3 RESULTS OF THE WATER-COLUMN TEST WITH *MYTILUS* SP.

The water-column test with *Mytilus sp.* was initiated on December 21, 2008 and was validated by 93.5% normal development in the control (Table 3-21). Dissolved oxygen, pH, and salinity fell within the test protocol limits throughout the duration of the 96-hour test (Tables 3-22 and 3-23). Temperature was above the recommended limits (16 ± 1 °C) for some of the test dilutions on Day 1 and Day 2. All of the dilutions that were above recommended limits were on the same shelf in the incubator. Given the high percentage of normal development in the test, reference, and control treatments, the slightly elevated temperature did not appear to have an effect on the outcome of the test.

The LC_{50} for the copper reference-toxicant test was calculated at 12.6 µg Cu/L, this value is within the control chart limits (3.6 – 18.4 µg Cu/L), indicating that the population of test organisms used in this test were similar in sensitivity to those previously tested at the NewFields laboratory.

The LC_{50} for the ammonia reference-toxicant test was 22.6 mg/L. Ammonia values in the test treatments were generally less than the LC_{50} , the highest measured concentration in the test samples was 26.5 mg/L. The ammonia values reported for Day 1 are considered suspect due to a malfunction with the ammonia probe caused by a puncture hole in the probe membrane. This puncture was found after the ammonia measurements were analyzed. The ammonia data are reported in Appendix C, and discussed in more detail in Section 4.

Mean percentage normal development in the 100% concentration for each of the test composites were 96.9% in Lower Comp, 63.2% in Area 1, 39.4% in Area 2, 16.1% in Area 4A, and 0% in Area 4B. The Site Water Control had 94.4% normal development and the Brine Control had 97.2% normal development. Mean normal development in the all other dilutions were above 93.4% for all of the samples except Area 4B where the 50% concentration produced a 60.7% normal development in the larvae.

The estimated EC_{50} for the composite treatments was >100% for Lower Comp and Area 1 and 87.3% Area 2, 74.6% Area 4A and 42.2% Area 4B. Statistical comparison to the control normal development for the 100% concentrations resulted in significant t-tests for Area 1 Comp, Area 2 Comp, Area 4A Comp, and Area 4B Comp.

Table 3-21. Normal Development Summary for *Mytilus* sp.

Sample ID	Concentration (%)	Mean Normal Development (%)	Standard Deviation	Significantly Less Than Control?
Control	0	93.5	3.5	--
Site Water	0	90.8	7.2	--
Brine Control	0	93.1	3.4	--
Area 1 Comp	1	96.0	4.1	--
	10	96.9	3.7	--
	50	96.2	4.8	--
	100	63.2	7.7	Yes
Area 2 Comp	1	99.4	0.9	--
	10	97.3	3.8	--
	50	96.5	3.5	--
	100	39.4	7.0	Yes
Area 4A Comp	1	96.8	3.3	--
	10	93.3	4.7	--
	50	96.3	4.2	--
	100	16.1	2.6	Yes
Area 4B Comp	1	98.6	3.0	--
	10	95.1	3.8	--
	50	60.7	8.4	--
	100	0.0	0.0	Yes
Lower Comp	1	98.8	1.8	--
	10	95.6	5.9	--
	50	93.4	7.2	--
	100	96.9	3.6	No

Table 3-22. Water Quality Summary for the Test with *Mytilus* sp.

Sample ID	Conc. (%)	Dissolved Oxygen (mg/L)			Temperature (°C)			pH (units)			Salinity (ppt)		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	0	7.8	7.0	8.7	16.3	15.1	17.1	7.8	7.5	8.0	31.0	31.0	31.0
Brine Control	0	7.4	7.2	7.6	16.5	15.2	17.2	7.8	7.6	8.0	31.0	31.0	31.0
Site Water	0	7.9	7.1	8.9	16.6	15.3	17.4	7.8	7.6	8.0	31.0	31.0	31.0
Area 1 Comp	1	7.7	7.1	8.2	16.4	15.2	17.2	7.9	7.6	8.0	30.7	30.0	31.0
	10	7.7	7.2	8.4	16.3	15.1	17.1	7.9	7.7	8.0	31.0	31.0	31.0
	50	7.6	7.2	8.0	16.4	15.2	17.2	8.0	7.9	8.1	30.7	30.0	31.0
	100	7.4	7.1	7.6	16.7	16.0	17.2	8.1	8.0	8.2	30.0	30.0	30.0
Area 2 Comp	1	7.7	7.1	8.3	16.4	15.5	17.1	8.1	7.9	8.2	30.7	30.0	31.0
	10	7.8	7.3	8.3	16.2	15.2	16.9	8.0	7.8	8.1	30.7	30.0	31.0
	50	7.5	7.1	7.9	16.4	15.5	17.0	8.1	8.0	8.2	30.7	30.0	31.0
	100	7.4	7.1	7.6	16.4	15.4	17.0	8.2	8.2	8.3	30.0	30.0	30.0
Area 4A Comp	1	7.5	6.9	8.4	16.9	15.1	17.9	7.9	7.9	8.0	30.7	30.0	31.0
	10	7.3	6.6	8.3	17.3	15.2	18.6	8.0	7.9	8.0	31.3	31.0	32.0
	50	7.2	6.7	8.0	18.3	15.3	20.4	8.1	8.0	8.2	31.0	31.0	31.0
	100	7.2	6.6	7.6	17.3	15.6	18.4	8.2	8.2	8.3	30.7	30.0	31.0
Area 4b Comp	1	7.4	7.0	8.2	17.2	15.2	18.5	8.1	7.9	8.2	30.7	30.0	31.0
	10	7.3	6.9	8.2	18.3	15.3	19.8	8.0	7.9	8.1	31.0	31.0	31.0
	50	7.2	6.9	7.9	17.0	15.1	18.3	8.2	8.1	8.2	30.7	30.0	31.0
	100	7.0	6.6	7.5	17.4	16.0	18.2	8.3	8.2	8.3	30.7	30.0	31.0
Lower Comp	1	7.3	6.7	8.2	18.2	15.5	19.8	8.1	8.1	8.1	31.0	31.0	31.0
	10	7.5	6.9	8.2	17.2	15.3	18.4	8.1	8.0	8.1	31.0	31.0	31.0
	50	7.4	7.0	7.9	17.1	15.1	18.4	8.0	8.0	8.0	31.0	31.0	31.0
	100	7.2	6.8	7.7	18.2	15.7	19.4	8.1	8.0	8.1	30.7	30.0	31.0

Table 3-23. Test Conditions for *Mytilus* sp.

Test Conditions: <i>Mytilus</i> sp.		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	5 weeks	
Test Species	<i>Mytilus</i> sp.- described as <i>M. galloprovincialis</i>	
Supplier	Carlsbad Aquafarms	
Date acquired	12/19/08	
Organism Acclimation/holding	2 days	
Age class	Larval	
Age of test animals	<4 hours	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	48-hour SPP	
Test dates	12/21/08 – 12/23/08	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 16 ± 1 °C	Achieved: 15.1 – 19.8°C
Test Salinity	Recommended: 31 ± 2 ppt	Achieved: 30 - 31 ppt
Test dissolved oxygen	Recommended: > 4.0 mg/L	Achieved: 6.6 – 8.9 mg/L
Test pH	Recommended: 8.0 ± 1	Achieved: 7.5 – 8.3
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: 4.4 %
Reference Toxicant LC50	12.4 µg Cu/L	
Acceptable Range	3.4 – 18.7 µg Cu/L	
Test Lighting	16- hours light, 8-hours dark	
Test chamber	1-L Glass Chamber	
Replicates/treatment	5	
Organisms/replicate	Stocking density = 383 embryos per chamber	
Exposure volume	10mL	
Feeding	None	
Water renewal	None	
Deviations	Temperature slightly out of range for a few samples target temperature range is 16 to 18 °C highest temperature was 18.3 °C	

3.8 BIOACCUMULATION TEST RESULTS

Assessment of bioaccumulation potential (BP) was determined by a 28-day exposure to each of the treatment samples and reference samples. The BP test was conducted with the polychaete, *Nephtys caecoides* and the clam, *Macoma nasuta*. Following the laboratory exposures, the test organisms were depurated for 24-hours and then placed in certified-clean glass jars and frozen. *M. nasuta* were depurated in clean seawater in the absence of sediment, and *N. caecoides* were depurated in clean sand. Tissues were sent via courier to the chemistry laboratory for analysis.

The 28-day bioaccumulation test was initiated on January 9, 2009. Tests were validated by 100% survival in control samples for *N. caecoides* and 94% control survival for *M. nasuta* (Table 3-24). All water quality parameters fell within the target limits throughout the duration of the 28-d test (Table 3-25 and Table 3-26). Survival in the reference and test sediment samples for *M. nasuta* ranged from 96% to 100% while survival for *N. caecoides* was between 82% and 98%; indicating sufficient tissue for chemical analysis.

Table 3-24. Survival Summary for *Nephtys caecoides* and *Macoma nasuta* Tests

Sample ID	<i>N. caecoides</i>		<i>M. nasuta</i>	
	Mean Survival (%)	Standard Deviation	Mean Survival (%)	Standard Deviation
Control	100	0.0	94	5.5
REF-Comp	90	4.6	96	5.5
REF-X	94	10.4	96	8.9
Area 1 Comp	98	5.4	100	0.0
Area 2 Comp	90	8.8	98	4.5
Area 4A Comp	82	6.4	96	8.9
Area 4B Comp	90	10.4	98	4.5
Lower Comp	92	7.5	96	8.9

Table 3-25. Water Quality Summary for the 28-Day Bioaccumulation Test

Sample ID	Dissolved Oxygen (mg/L)			Temperature (°C)			Salinity (ppt)			pH		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	7.4	6.3	8.1	15.8	15.1	16.4	31.2	31	32	7.6	7.4	7.8
REF-Comp	7.5	6.8	8.3	15.8	15.1	16.2	31.2	31	32	7.7	7.5	7.8
REF-X	7.7	6.9	8.2	15.7	15.0	16.4	31.2	30	32	7.7	7.5	7.9
Area 1 Comp	7.3	6.2	8.0	15.8	15.0	16.4	31.2	31.0	32.0	7.6	7.2	7.8
Area 2 Comp	7.3	6.5	8.0	15.7	15.1	16.6	31.1	30.0	32.0	7.6	7.2	7.8
Area 4A Comp	7.3	6.3	7.9	15.7	15.1	16.4	31.2	31.0	32.0	7.7	7.3	7.8
Area 4B Comp	7.4	6.7	8.1	15.8	15.1	16.6	31.3	31.0	32.0	7.7	7.3	7.8
Lower Comp	7.4	6.7	8.1	15.8	15.1	16.6	31.3	31.0	32.0	7.7	7.3	7.8

Table 3-26. Summary of Test Conditions for 28-day Bioaccumulation Test

Test Conditions for Bioaccumulation Test		
Date sampled	11/17/08	
Date received	11/28/08	
Sample storage	4°C, dark	
Weeks of holding	1 week	
Acclimation of test treatment	3 weeks under static renewal with raw seawater	
Test Species	<i>Macoma nasuta</i> and <i>Nephtys caecoides</i>	
Supplier	J & G Gunstone provided clams and John Brezina provided worms	
Date acquired	1/8/09 and 1/7/09	
Organism acclimation/holding time	2 days	
Age class	Adult	
Test Procedures	ITM	
Regulatory Program	ITM	
Test location	NewFields Northwest Laboratory	
Test type/duration	28-Day Bioaccumulation	
Test dates	1/9/09 – 2/6/09	
Control water	0.2µm-filtered North Hood Canal sea water	
Test temperature	Recommended: 15 ± 1 °C	Achieved: 15.0 – 16.6 °C
Test Salinity	Recommended: 32 ± 2 ppt	Achieved: 30 - 32 ppt
Test dissolved oxygen	Recommended: > 4.5 mg/L	Achieved: 6.3 – 8.3 mg/L
Test pH	Recommended: 7.8 ± 0.5	Achieved: 7.2 – 7.9
Control performance standard	Recommended: Control ≤ 10% mortality	Achieved: <i>M. nasuta</i> – 6% <i>N. caecoides</i> – 0%
Test Lighting	16- hours light, 8-hours dark	
Test chamber	10 Gallon Glass Aquarium	
Replicates/treatment	5	
Organisms/replicate	10 clams, 25 worms	
Exposure volume	5 cm of sediment, 30 L seawater	
Feeding	None	
Water renewal	Flow-Through	
Deviations	None	

3.9 TISSUE CHEMICAL ANALYSIS

Bioaccumulation tests were conducted using the test composites, the reference composite and the individual samples from the five reference locations. Based on the sediment chemistry, tissues from the bioaccumulation exposures were analyzed for mercury and lipids. Mean concentrations found the test treatments and the reference treatments are presented in Table 3-27. Mercury concentrations ranged from 0.016 ug/g (REF-Comp) to 0.213 ug/g (Lower Comp) in the tissues of *M. nasuta* and from 0.008 ug/g (REF-Comp) to 0.027 ug/g (Lower Comp) in the tissues of *N. caecoides*.

Table 3-27. Results of Mercury Analysis of Tissues

Sample ID	Rep	<i>M. nasuta</i>		<i>N. caecoides</i>	
		Lipid (%)	Mercury (µg/g wet weight)	Lipid (%)	Mercury (µg/g wet weight)
Area 1 Comp	1	0.84	0.0326	1.01	0.00947
	1 dup	NA	NA	1.02	NA
	2	0.81	0.0291	0.64	0.00782
	3	0.78	0.0268	0.57	0.00770
	4	0.54	0.0244	0.45	0.00746
	5	0.83	0.0248	1.10	0.0101
Area 2 Comp	1	0.92	0.0518	0.83	0.00961
	2	0.81	0.0534	0.72	0.0135
	3	0.70	0.0521	0.50	0.00878
	3 dup	NA	NA	0.48	NA
	4	0.81	0.0404	0.50	0.0135
	5	0.82	0.0666	1.02	0.0135
Area 4A Comp	1	0.92	0.0388	0.80	0.00941
	2	0.80	0.0459	0.44	0.00913
	3	0.76	0.0424	0.93	0.0102
	4	0.73	0.0372	1.09	0.0109
	5	0.57	0.0311	0.53	0.00902
Area 4B Comp	1	1.11	0.0329	0.66	0.00902
	2	0.73	0.0512	0.58	0.00922
	3	0.62	0.0374	0.46	0.0102
	4	0.87	0.0471	0.59	0.0102
	5	0.89	0.0404	0.55	0.00879
Lower Comp	1	0.81	0.206	0.47	0.0269
	1	NA	0.220	NA	NA
	2	1.03	0.235	0.73	0.0323
	3	0.80	0.242	0.48	0.0276
	4	0.86	0.199	0.83	0.0251
	5	0.39	0.186	0.45	0.0246
	5 dup	0.36	NA	NA	0.0248

Sample ID	Rep	<i>M. nasuta</i>		<i>N. caecoides</i>	
		Lipid (%)	Mercury ($\mu\text{g/g}$ wet weight)	Lipid (%)	Mercury ($\mu\text{g/g}$ wet weight)
REF-Comp	1	0.96	0.0186	0.72	0.00787
	2	0.83	0.0159	0.75	0.00826
	3	0.95	0.0141	0.80	0.00828
	4	0.80	0.0155	0.77	0.00752
	4 dup	NA	NA	NA	0.00750
	5	0.74	0.0148	0.47	0.00764
REF-01	1	0.93	0.0168	0.59	0.00801
REF-02	1	0.93	0.0133	0.90	0.00679
REF-03	1	0.80	0.0140	0.49	0.00879
REF-04	1	0.77	0.0159	0.75	0.00791
	dup	NA	0.0161	NA	NA
REF-05	1	0.71	0.0127	0.75	0.00799
	dup	0.70	NA	NA	NA
Control	1	0.89	0.0169	0.79	0.00666
	2	0.77	0.00950	0.83	0.00718
	3	0.68	0.00999	0.43	0.00677
	4	0.90	0.0108	0.89	0.00700
	5	0.99	0.0121	0.98	0.00730

4 DISCUSSION

The objective of this sampling and analysis program was to characterize the dredged materials from Douglas Harbor's four dredged material management units (Areas 1, 2, 4A and 4B). The primary disposal option for the dredged material from Douglas Harbor is inland aquatic disposal at the Gastineau Channel Dredged Material Disposal Site. Decision criteria for the evaluation of disposal suitability followed guidelines set forth in the Inland Testing Manual (ITM; USACE/USEPA 1998), and where appropriate, the PSDDA Users Manual (USACE 2008). The following discussion evaluates the physical, chemical, and biological data for each composite relative to the proposed inland aquatic disposal option. These documents rely on the toxicological responses of test organisms exposed to dredged material during removal and disposal through the water column and sediment that has been placed at the disposal site. Additionally, the ecological and human health considerations for project bioaccumulation of contaminants of concern are addressed using these documents and consensus guidance provided by the agencies earlier this year (USACE 2008; USACE/EPA 1998; State of Alaska Division of Public Health. 2007; and supplemental guidance provided by ADEC in their email 1/12/2009).

4.1 BENTHIC TEST SUMMARY FOR *AMPELISCA ABDITA*

The benthic test with *A. abdita* was conducted using the standard testing protocol and a suggested adjustment to that protocol to acclimate the sediment to appropriate biological conditions. As discussed during the preliminary Sampling and Analysis Planning meeting (November 5, 2008) this procedure has been successfully applied to remove contributing factors to adverse biological effects that are not associated with the persistent chemicals of concern. Some of those interfering contributing factors can be divided into three groups: persistent features, non persistent features, and non matrix characteristics. Persistent features do not easily change through time (e.g. sediment grain size, total organic carbon content, or water hardness for freshwater testing). Conversely, less persistent features produce effects that are time dependent and include such characteristics as ammonia or sulfide concentration, the quality of the organic carbon in the sediment, the pore water salinity and whether the sediment or soil is an appropriate habitat or must be acclimated prior to testing conditions. Other factors that are not physical or chemical include the selection of the appropriate test organism, the health of the test organism prior to and during testing, test organism acclimation and handling techniques. For a complete description of confounding factors and their application to sediment evaluations see Word et al. 2005.

The benthic test with *A. abdita* (Jan. 2009) was conducted using sediment both acclimated and unacclimated sediment for Area 4B, the Lower Composite sample (Lower Comp), the five individual reference stations, and the Reference -Composite (REF-Comp). Materials from Areas 1, 2, and 4A were tested only on acclimated sediment. The decision to perform both standard and acclimated sediment testing was based on providing appropriate biogenic testing conditions so that the effects of chemical contaminants could be separated from other contributing factors. The Lower Comp's characteristics that suggested this need as it was sandy with low total organic carbon content and had been buried at depths beyond the reach of benthic organisms for long periods of time. The Area 4B composite had relatively high levels of the less persistent ammonia and sulfides in their pore waters. Acclimation of these samples is expected to result in establishing a sediment that contained appropriate microorganisms that would handle test organism waste materials without permitting development of toxicologically important levels of ammonia or sulfides and would also modify some of the TOC in the sample to provide more biogenically available food for the test organisms (Spotte, 1992; Word et al, 2005). The reference area

sediments were acclimated in a similar manner as the test samples as a control measure to examine the effect of acclimation on samples that are expected to be acceptable.

The quantity of total organic carbon in the Lower Comp sediment (measured in the lower cores of each Area 1, 2, 4A, and 4B ranged from 0.047 to 0.069%) could have influenced the outcome of the test if some fraction of the total organic carbon was not available as a food source for the amphipods. They would then be stressed based on starvation. This sediment was acclimated by exposure to raw seawater in a carefully replaced static renewal system for three weeks prior to use in the amphipod test. This process allowed natural microbial populations to establish using the existing small amount of TOC to create a higher quality source of food for the amphipods.

The acclimation process for Area 4B Comp followed a different acclimation procedure to reduce any adverse influence from elevated pore water ammonia concentrations. The sediment was layered into the test chambers one week prior to testing and placed under test conditions. It has been established that sediment or soils placed into conditions that are not similar to their original source require acclimation to those conditions prior to successful testing of marine organisms (Spotte 1992). The typical ammonia pattern for sediment that is not acclimated prior to testing starts with relatively low concentrations of total ammonia in the overlying water, with increases in the ammonia concentrations after the first few days and subsequent decreases in ammonia concentrations after the microbial community is established.

The ammonia production cycle can be missed if ammonia is only measured at the start and end of a test. The lack of acclimation of sediment to test conditions has been shown to have an extensive influence on the survival of test organisms with as much as an 80% increase in toxicity when the sediments are not acclimated prior to the introduction of test organisms (Word *et al.* 2005).

The total ammonia concentrations for Area 4B Comp unacclimated and acclimated sediment are summarized in Table 4-1. The concentration of ammonia in the pore water was reduced by the acclimation process to below amphipod threshold levels of 20 mg/L.

Table 4-1. Total Ammonia Concentration Measured in Area 4B Comp, *Ampelisca abdita* Test

Treatment	Total Ammonia (mg/L) Test Day 0 Pore water	Total Ammonia (mg/L) Test Day 10 Pore water
Area 4B Comp Unacclimated	45.9	6.77
Area 4B Comp Acclimated	6.22	6.08

The acclimation of sediment composites did positively affect the survival of *A. abdita* in both composites. Survival in the Area 4B Comp increased from 87% to 94% and survival in the Lower Comp increased from 76% to 94%. The acclimation process did not change the results for the reference samples. Survival in the REF-Comp was 93% (unacclimated) and 90% (acclimated). Mean survival in the individual Reference (REF-X) samples was 95% (unacclimated) and 96% (acclimated). The water quality measurement of DO, pH, salinity, and temperature remained within target limits throughout the duration of the test.

For validated benthic toxicity tests, the ITM evaluation criteria for benthic toxicity are defined as: statistically significant increase in toxicity relative to the reference and increased mortality >20% (acceptable limit for *A. abdita*) above the reference survival. Under the PSDDA program, a test treatment will fail if mean mortality in the test is >20% more than the mean mortality in the appropriate control sediment or more than 10% above the appropriate reference and the difference is statistically significant ($p \leq 0.05$). Table 4-2 provides a summary of the amphipod test relative to the performance criteria.

Mortality in sediments from the Douglas Harbor were not statistically significantly higher in mortality than the reference sediment, and no mortalities exceeded the numerical criteria relative to the reference, therefore all test treatments pass the performance criteria in the ITM and the PSDDA methods (Table 4-2).

Table 4-2. Performance Criteria Comparison for *Ampelisca abdita*

Treatment	Mean Mortality (%)	Statistically greater than REF-Comp?	M _T -M _C	M _T -M _R	Pass ITM	Pass PSDDA
Control	9	---	---	---	---	---
REF-Comp	7	---	---	---	---	---
REF-Comp Acclimated	10	---	---	---	---	---
REF-X	5	---	---	---	---	---
REF-X Acclimated	4	---	---	---	---	---
Area 1 Comp	8	No	-1	1	Yes	Yes
Area 2 Comp	8	No	-1	1	Yes	Yes
Area 4A Comp	10	No	1	3	Yes	Yes
Area 4B Comp	13	No	4	6	Yes	Yes
Area 4B Comp Acclimated	6	No	-3	-4	Yes	Yes
Lower Comp	24	Yes	15	17	Yes	Yes
Lower Comp - Acclimated	6	No	-3	-4	Yes	Yes

4.2 BENTHIC TEST SUMMARY FOR *NEANTHES ARENACEODENTATA*

The sediment composites were not acclimated for the *N. arenaceodentata* test based on guidance provided in the DMMP clarification paper *Ammonia and Sulfide Guidance Relative to Neanthes Growth Bioassay (6/15/04)*. No effects on mortality were observed with bulk sediment ammonia values of ≤ 115 mg/Kg and total sulfides of ≤ 3.4 mg/L in the overlying water. The decision not to acclimate was confirmed by $> 84\%$ survival in all of the test treatments. The water quality measurements remained within target limits throughout the duration of the test and a summary of the ammonia and sulfide values for each composite are summarized in Table 4-3.

Table 4-3. Summary of Total Ammonia and Sulfide Concentrations for the *Neanthes arenaceodentata* Benthic Test

Treatment	Total Ammonia Overlying Water (mg/L) Day 0	Total Sulfide Overlying Water (mg/L) Day 0
Control	<0.5	0.141
REF Comp	<0.5	0.092
Area 1 Comp	0.702	0.016
Area 2 Comp	1.12	0.003
Area 4A Comp	1.82	0.009
Area 4B Comp	2.76	0.063
Lower Comp	<0.5	0.047

The ITM evaluation criteria for benthic toxicity are defined as: significant toxicity relative to the reference and mortality >10% above the reference survival. The PSDAA Users Manual (July 2008) does not provide performance criteria for the *N. arenaceodentata* 10-day test. Although the response for the lower composite survival meets the >10% portion of the criteria, the replicate data for that composite are sufficiently variable to not be statistically significant. Table 4-4 provides a summary of the polychaete test relative to the performance criteria.

Table 4-4. Performance Criteria Comparison for *N. arenaceodentata*

Treatment	Mean Mortality (%)	Statistically greater than REF-Comp?	M _T -M _C	M _T -M _R	Pass ITM
Control	0	---	---	---	---
REF-Comp	4	---	---	---	---
REF-X	8	---	---	---	---
Area 1 Comp	4	No	4	0	Yes
Area 2 Comp	12	No	12	8	Yes
Area 4A Comp	8	No	8	4	Yes
Area 4B Comp	0	No	0	-4	Yes
Lower Comp	16	No	16	12/8	Yes*

*Although the mean mortality is greater than 10% for the lower composite relative to the REF-Comp, replicate variability is sufficiently large to not be statistically significant. ITM requires that both a statistically significant increase in mortality plus an effect greater than 10% be required before the differences are biologically significant. Additionally, Comparison to REF-X mean is neither statistically significant nor >10% increase in mortality.

4.3 WATER-COLUMN SUMMARY

No significant toxicity was observed in the water column tests with *M. beryllina* or *A. bahia*, all test concentrations had greater than >96% survival and no statistically significant differences were observed in the 100% elutriate samples when compared to the control survival. In the larval development test for *Mytilus* sp., statistically significant differences were observed between the 100% elutriate concentration and the 0% elutriate (site water) for treatments Area 1, Area 2, Area 4A and Area 4B. The calculated EC₅₀ for each test composite is summarized in Table 4-5.

Table 4-5. Calculated EC50 Values for the *Mytilus* sp. Test

Calculated EC ₅₀	Area 1	Area 2	Area 4A	Area 4B	Lower Comp
	>100%	87.3	74.6	42.2	> 100 %

Table 4-6 provides the measured ammonia values in the elutriate concentrations. The highest ammonia values were observed in the 100% elutriates from Area 1 Comp and Area 4B Comp. An ammonia reference toxicant test was conducted along with the elutriate test. The measured ammonia concentrations in the reference toxicant test were higher than expected based on nominal concentrations. The calculated EC₅₀ from this reference toxicant test was 22.6 mg/L and the lowest observable effects concentration was 19.7 mg/L. These values are higher than other ammonia reference toxicant tests shown in Figure 4-1.

Table 4-6. Relative Concentrations of Ammonia Measured in the Reference Toxicant and Elutriate Test for *Mytilus* sp.

Treatment	Elutriate Conc. (%)	Measured Ammonia Concentration Day 0 (mg/L)	Measured Ammonia Concentration Day 2 (mg/L)	LOEC from Ammonia Ref Tox (mg/L)	EC ₅₀ from Ammonia Ref Tox (mg/L)
Control		2.03	<0.5	19.7	22.6
Site Water		<0.5	<0.5		
Brine Control		5.6	<0.5		
Area 1 Comp	1	<0.5	NM		
	10	10.8	<0.5		
	50	15.5	1.01		
	100	21.6	2.76		
Area 2 Comp	1	1.84	NM		
	10	4.40	<0.5		
	50	12.3	1.32		
	100	18.5	2.85		
Area 4A Comp	1	1.41	NM		
	10	3.90	<0.5		
	50	12.1	1.43		
	100	15.7	3.91		
Area 4B Comp	1	1.25	NM		
	10	5.82	<0.5		
	50	17.1	2.29		
	100	26.2	5.14		
Lower Comp	1	0.794	NM		
	10	1.06	NM		
	50	3.66	NM		
	100	5.47	<0.5		

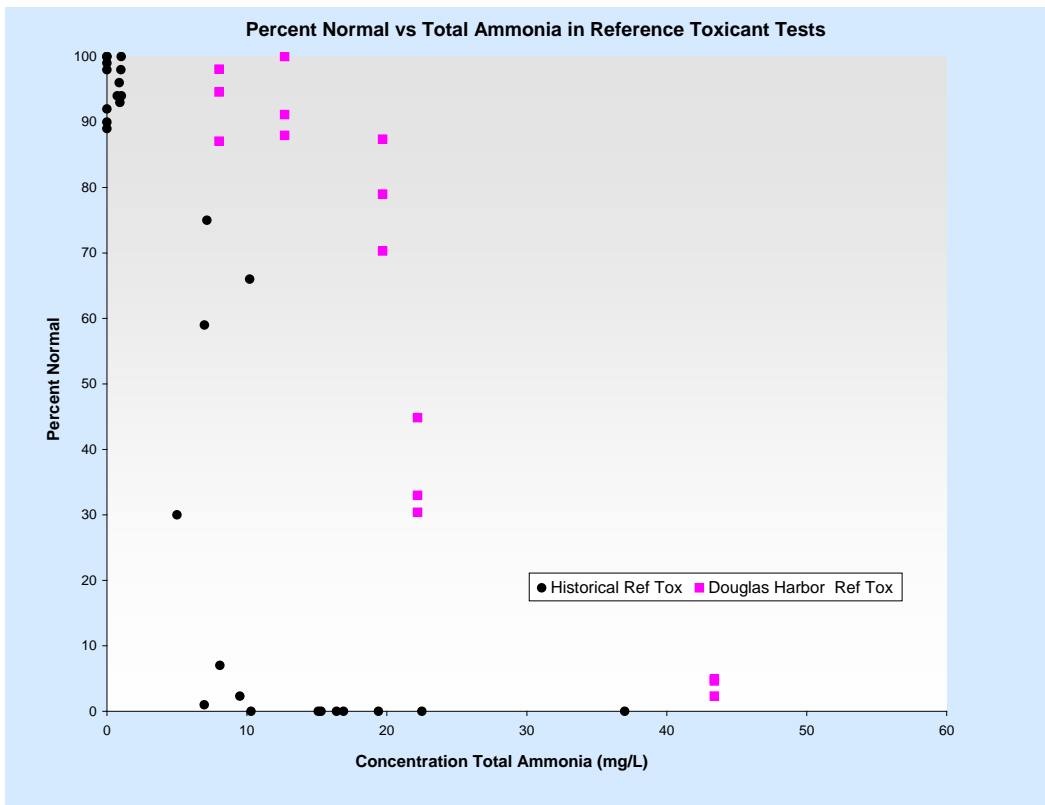


Figure 4-1. Comparison of Douglas Harbor Reference Toxicant to Historical Reference Toxicant Tests (W. Gardiner, personal communication)

The ammonia values measured on Day 0 in the elutriate test and the ammonia values for the reference toxicant test were measured on the same day using the same ammonia probe. The higher than expected ammonia readings obtained for the reference toxicant test and the higher EC_{50} for the larval test raises concerns that the ammonia meter was not functioning properly during that time. The lab technician did find a hole in the membrane and replaced the membrane probe. The ammonia data for this test should be used only to *estimate* the relative contribution of ammonia to the test results. However, the ammonia concentrations in the 100% elutriates were above the lowest observable effects concentration of 19.7 mg/L in the reference toxicant test indicating that toxicity of these concentrations may in part be related to ammonia.

The contribution of ammonia to the overall toxicity of the elutriate preparation is not part of the decision criteria for suitability of the sediment for disposal. For the water-column tests, the performance criteria from the ITM is that the 100% elutriate concentration is not statistically higher than the 0% elutriate concentration and that the dissolved and suspended contaminants, after allowance for initial mixing, do not exceed 0.01 of the toxic concentration (expressed as the EC_{50} or LC_{50}) beyond the boundaries of the mixing zone. The limiting permissible concentration was determined using the Short-Term Fate (STFATE) model as summarized below.

4.3.1 LIMITING PERMISSIBLE CONCENTRATION DETERMINATION

For sediment to be considered suitable for aquatic disposal the mean percentage survival or normality in the water column 100% concentrations must not be statistically significantly different than the 0% SPP treatment *and* the modeled concentration at the edge of the disposal site must not exceed Limiting Permissible Concentration (LPC). The STFATE model for dredged material disposal was used to determine whether water quality criteria would be violated during the disposal of sediments at the Gastineau Channel Disposal Site.

The LPC for the water column bioassays is one-hundredth of the acutely toxic concentration (the LC₅₀ or EC₅₀) of dredged material in the water column after the initial 1-hour mixing period. The STFATE model used for this determination is based on sediment characteristics (grain size, percent solids, and toxicity), physical oceanographic conditions at the site, the size of the designated site, and the volume of sediment to be discharged (USEPA/USACE 1998, Appendix C).

Based on the results of the larval test, the LPC for the test composites was calculated as 42.2% concentration for Area 4B Upper. This was the lowest LC₅₀ (most toxic) for any of the sites and also the finest sediment. Using the STFATE model, the LPC was calculated for the Gastineau Channel Disposal Site, a summary of the input parameters and model outputs are shown in Table 4-7. The maximum concentration at the site boundary after one hour was calculated to be 0.347%. This value is below the LPC for each of the test composites.

Table 4-7. Input Parameters to STFATE.

Calculation of Limiting Permissible Concentration Using STFATE (Ver 5.01)	
Model Input	Gastineau Channel
Mixing Area	
Depth of site (ft)	120
Width of site (Northeast to Southwest, ft)	375
Length of site (Northwest to Southeast, ft)	600
Area of site (sq ft)	225,000
Volume of disposal vessel (cu yd)	500
Length of simulation (hrs)	1
Composition of material	
Solids (%)	64.9
Sand (%)	10.9
Silt (%)	65.1
Clay (%)	21.7
Fluids (%)	35.1
Density of water (g/cc)	1.02
Water Quality Results	
Lowest LC ₅₀ or EC ₅₀ (%)	42.2
Limiting Permissible Concentration (%) = 0.01 of LC ₅₀ or EC ₅₀	0.422
Maximum concentration within mixing area during simulation (%)	0.455
Maximum concentration within mixing area at end of simulation (%)	0.0245
Maximum concentration outside disposal site during simulation (%)	0.347
Maximum concentration disposal site at end of simulation (%)	0.0245
Water Quality Criteria Violated?	No

4.4 BIOACCUMULATION SUMMARY

The 28-day bioaccumulation test was conducted using *Macoma nasuta* and *Nephtys caecoides*, two species recommended in the ITM. The ITM protocol for conducting the bioaccumulation test is 28-days. This test has been established and approved for use throughout the United States for a variety of contaminants including metals. For some organic chemicals that have a slower rate of uptake to a state of tissue equilibrium there are application factors applied to these 28-day uptake values. Mercury is not one of these; therefore the 28-day exposure period is the default time frame for ITM assessments. In the absence of a regional guidance manual, the federal manual guidance was used for this project. The rationale for the 28-day testing period is on page 6-3 through 6-5 of the ITM and summarized below:

- “The time to reach or approach steady-state varies among different compounds and, to a lesser extent among different species. Test designs that assure that steady state has been attained require a large number of samples and substantial expense. As a cost-effective compromise, it is recommended that a 28-day exposure be used for the “standard” bedded sediment bioaccumulation test for neutral organics and metals.”
- “Where it is desirable to know the steady-state concentration of neutral organic compounds as, for example, comparison to an FDA action level, fish advisory or similar numerical values, the following procedure is recommended. The log K_{ow} of the neutral organic compound of concern should be compared with the log K_{ow} in Figure 6-1 (from the ITM 1998) and will indicate the proportion of steady-state concentration (C_{ss}) expected in 28 days based on empirical evidence. This will allow estimation of the steady-state value from the 28-day laboratory exposure data using a steady-state correction factor. The correction factor is the reciprocal of the decimal fraction indicating the proportion of C_{ss} expected in 28 days.”

The octanol/water partition coefficient (K_{ow}) for methyl mercury was not provided in the ITM, therefore a list of published K_{ow} along with their citations is provided in Table 4-8.

Table 4-8. Octanol Water Partition Coefficients for Mercury

Kow	Citation
1.7	Mason et al. 1995
1.5	National Academic Press 2000

Figure 4-2 shows that Log K_{ow} values below 4.25 reach steady state within the 28-day exposure period. The low Log K_{ow} for methyl mercury suggests that a 28-day exposure is an appropriate amount of time to for any methyl mercury present in the bioaccumulation organisms to reach steady state.

Extending the bioaccumulation test beyond 28 days may have resulted in higher mortality of the test organisms due to starvation, especially for sediment with a low total organic carbon content, for example Lower Comp. This composite required acclimation (Section 2.5.1).

Discussions with CBJ, PND, and the regulatory agencies led to the acceptance the 28-day bioaccumulation protocol established by the ITM for use on Douglas Harbor sediment. Using this

established method provided a robust *scientifically defensible* data set for making decisions regarding appropriate placement of dredged material from Douglas Harbor.

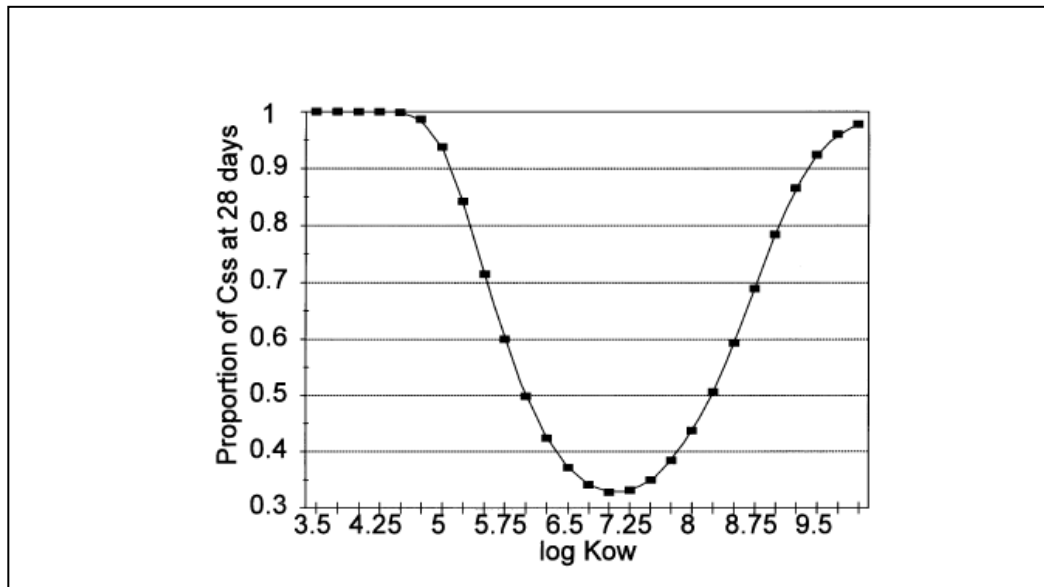


Figure 4-2. Plot of KOW and Steady State at 28-Days (USEPA/USACE 1998)

No significant toxicity was observed in the bioaccumulation tests performed on Douglas Harbor sediments. Survival in all treatments was 84% or greater, providing adequate tissue mass for chemical analyses.

Statistical analysis of test treatments compared to reference treatments showed that mercury concentrations in all test treatments were statistically significantly higher than in the REF-Comp with the exception of Area 1 Upper for *N. caecoides* (Table 4-9). The 95% upper confidence limit (UCL) for mercury in each of the composite tissue samples of *M. nasuta* and *N. caecoides* were below the 0.32 ppm wet weight threshold concentration provided by ADEC as a consensus agreement for consumption of fish and shellfish for Alaskans (Table 4-9, Figure 4-3).

In 2007 the State of Alaska Division of Public Health published the Epidemiology Bulletin Volume 11, Number 4 entitled, “*Fish Consumption Advice for Alaskans: A Risk Management Strategy to Optimize the Public’s Health.*” This Bulletin includes information about mercury in fish in Alaska and gives recommended consumption allowances. The Bulletin describes an EPA screening value for unlimited consumption defined as over 16 meals per month. For 16 meals per month a monthly consumption allowance for fish of 0.32 ppm wet weight of total mercury (assumed that all mercury is methyl mercury). The consensus agreement provided by ADEC considers the 0.32 ppm as the tissue concentration number that should be used based on the Alaska fish advisory. In all cases the concentration of total mercury after the 28-day exposure period was below this consensus value for both species. In fact all of the concentrations obtained were well below the 0.15 mg/kg wet weight value except the clam for the lower composite for the unrestricted consumption value provided by the Alaska Department of Health and Social Services.

Table 4-9. Summary Statistics for Tissue Concentrations of Mercury

Composite Sample	Prob Normal ($\alpha=0.01$)	Prob Equal Variance ($\alpha=0.10$)	Prob Normal Log Transform ($\alpha=0.01$)	Prob Equal Variance Log Transform ($\alpha=0.10$)	Mean (ug/g)	Sig. Greater Than Reference ($\alpha=0.05$)	95% UCL	UCL Greater Than ADEC Action Level (0.32 ug/g)
<i>Macoma nasuta</i>								
Area 1	ANOVA / One-tailed LSD Log transformed Data	<0.001	0.652	0.882	0.027	Yes	0.031	No
Area 2					0.052	Yes	0.058	No
Area 4A					0.039	Yes	0.043	No
Area 4B					0.041	Yes	0.046	No
Lower					0.213	Yes	0.237	No
REF-Comp					0.016	--	--	
<i>Nephtys caecoides</i>								
Area 1	One-tailed T-test Log transformed Data	0.006	0.333	<0.001	0.008	No	0.010	No
Area 2					0.012	Yes	0.014	No
Area 4A					0.010	Yes	0.010	No
Area 4B					0.009	Yes	0.010	No
Lower					0.027	Yes	0.030	No
REF-Comp					0.008	--	--	

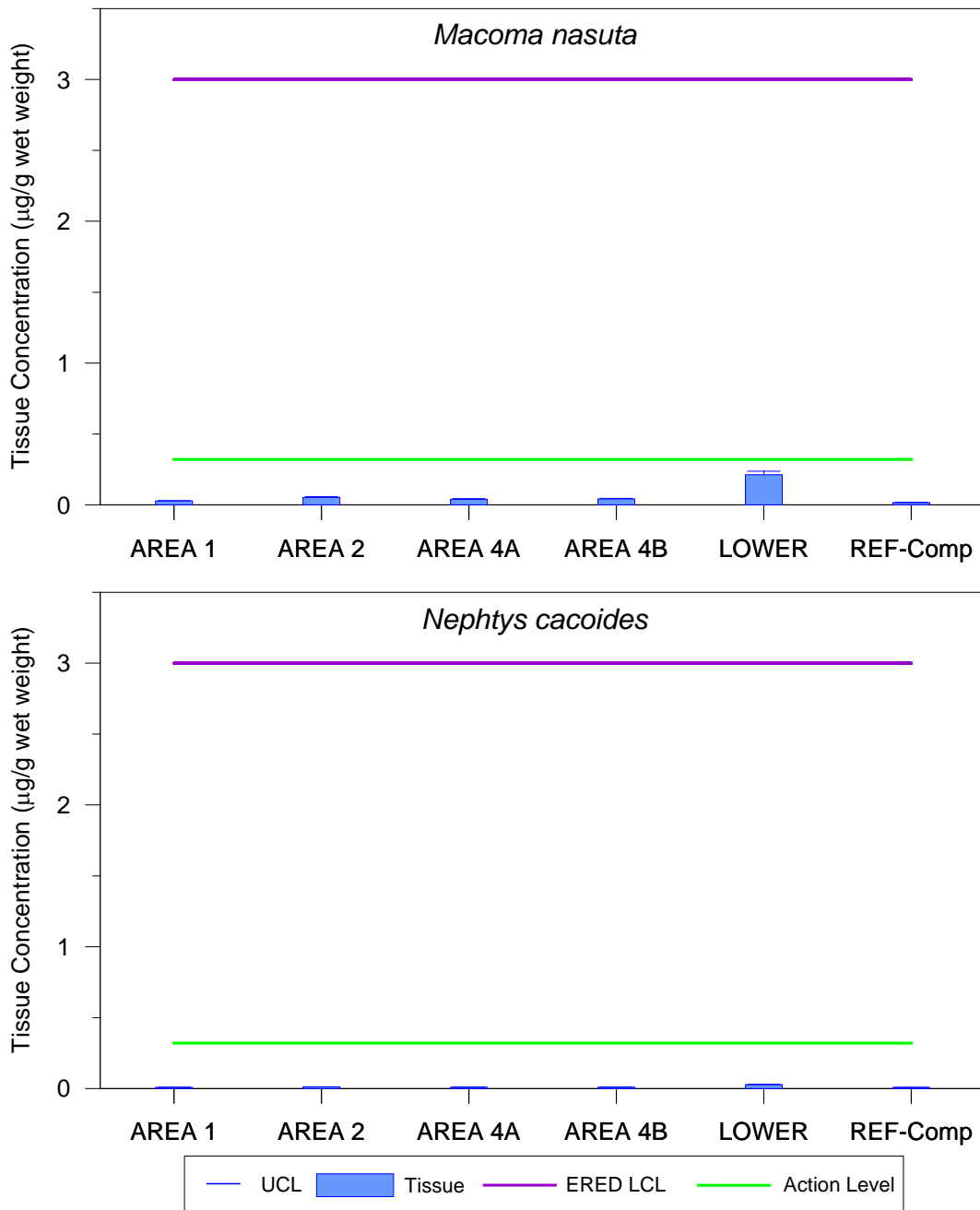


Figure 4-3. Plot of Tissue Concentrations compared to 0.32 ppm Project-Specific Action Level and ERED Lower Confidence Limit.

Bioaccumulation evaluations also examine the potential for adverse effects to organisms living at the disposal site. The tissue concentrations of the test organisms are compared to effects based values that have been developed for each chemical of concern. The Environmental Residue Effects

Data base (ERED; USACE/USEPA 2008) that is annually maintained by USACE – ERDC contains approximately 14,000 pairs of chemical specific tissue burdens to adverse biological effects that have been extracted from the scientific peer reviewed literature. In the case of mercury, the effects include development, growth, mortality and reproductive end-point evaluations. For marine organisms the most sensitive end-point is growth and the 95% LCL is ~ 3 mg/kg wet weight. The data provided by the bioaccumulation testing demonstrates that organisms that directly feed on dredged sediment from Douglas Harbor, Alaska will accumulate mercury to levels that are at least a factor of 10 below these ecological risk benchmarks.

4.5 CONCLUSIONS

The results of the Tier III/IV evaluation for Douglas Harbor included sediment chemistry, biological testing, and bioaccumulation testing. The results were compared against ITM performance criteria and PSDDSA (Users Manual) where appropriate. A summary of the findings is presented in Table 4-11 and the following paragraphs:

Mortality in the benthic amphipod and the polychaete tests was not statistically greater than in the reference and did not exceed mortality in the reference sediment by at least 10% (polychaete) or 20% for amphipod, *Ampelisca abdita* (Swartz et al, 1985; Mearns et al, 1986; SAIC, 1992 a,b); the ITM performance criteria. Using the PSDDA criteria for the amphipods, no mean test mortality was greater than 20% over the mean negative control response, and mean test mortality was not greater than 10% (dispersive) or 30% (non-dispersive) over the mean reference sediment response or statistically significant compared to reference ($\alpha = 0.5$).

The results of the 100% dredged material elutriate toxicity in the larval water column tests were statistically higher than in the dilution water. Modeling of these water column effects were demonstrated to not have effects outside of the dredged material disposal site (STFATE). The dredged material is therefore not predicted to be acutely toxic to water-column organisms and the concentration of dissolved and suspended contaminants, after allowance for initial mixing, does not exceed 0.01 of the toxic concentration expressed as the EC or LC₅₀, beyond the boundaries of the mixing zone. Therefore the dredged material is predicted not to be acutely toxic to water column organisms.

Bioaccumulation data were evaluated based on two criteria. First the concentration of bioaccumulation of a specific contaminant in dredged material exposed organisms is compared to a numerical effect limit, such as a Food and Drug Administration action level or in this case the Alaska fish advisory. If the concentration of a contaminant in a dredged material exposed organism exceeds a numerical limit, there is the potential for the dredged material disposal to have an "unacceptable adverse effect." If it does not, or there is no numerical limit, a second level of evaluation is undertaken which involves a statistical comparison of the bioaccumulation response of animals exposed to the dredged material to that of animals exposed to the reference sediment. When a statistically significant comparison is found, then a number of evaluation factors are considered to determine whether or not dredged material disposal would be predicted to result in an "unacceptable adverse effect"; including consideration of the magnitude of bioaccumulation and the toxicological significance of the bioaccumulated contaminants (USEPA/USACE 1991 and 1998).

The results of the bioaccumulation test were compared to the ITM criteria and also the PSDDA (Users Manual) criteria. The mean tissues concentration in all of the test and reference treatments were below the FDA action level of 1.0 ppm wet weight and also below the project specific target

level of 0.32 ppm wet weight. All of the test composites were statistically significantly higher than the reference composite with the exception of Area 1 Upper for *N. caecoides*.

There are limitations regarding the use of the bioaccumulation guidance, the small number of published action limits available compared to the large number of contaminants commonly present in freshwater and marine sediments and uncertainties involved in using qualitative/subjective evaluation factors. The USACE Environmental Residue-Effects Database (USACE/USEPA 2008) was developed to reduce the level of uncertainty associated with interpreting bioaccumulation data for the purpose of making regulatory decisions regarding dredged material.

The ERED database was queried for all potential ecological effects resulting from mercury exposure. The output in the form of a graph (Figure 4-4) shows that all of the published effects related to mercury are at or above 3 ppm. The most sensitive assessment end-point for mercury in marine organisms is growth and its 95% LCL is ~3 mg/kg (wet weight). The highest tissue concentration reported was 0.242 ppm (Lower Comp Rep 3) suggesting that ecological effects are not likely to be observed by organisms exposed to sediment from Douglas Harbor, Alaska when placed at the Gastineau Channel Dredged Material Disposal Site.

Table 4-10. Summary Results for Douglas Harbor Dredged Material Evaluation

Summary Results	Area 1	Area 2	Area 4A	Area 4B	Area 4B Acclimated	Lower Comp	Lower Comp Acclimated
Benthic (% survival)	<i>Douglas Harbor composites pass ITM/ PSDDA Performance Criteria</i>						
<i>A. abdita</i>	92	92	90	87	94	76	94
<i>N. arenaceodentata</i>	96	88	92	100	NA	84	NA
Water-column (LC₅₀ or EC₅₀)	<i>Water Quality Criteria Pass (STFate Model)</i>						
<i>A. bahia</i>	>100%	>100%	>100%	>100%	NA	>100%	NA
<i>M. beryllina</i>	>100%	>100%	>100%	>100%	NA	>100%	NA
<i>Mytilus. Sp.</i>	>100%	87.3	74.6	42.2	NA	> 100 %	NA
Mean Mercury Conc. (ppm)	<i>Human Health Action level is 0.32 ppm and Ecological Health is 3.0 ppm</i>						
<i>M. nasuta</i>	0.027	0.052	0.039	0.041	NA	0.213	NA
<i>N. caecoides</i>	0.008	0.012	0.010	0.009	NA	0.027	NA

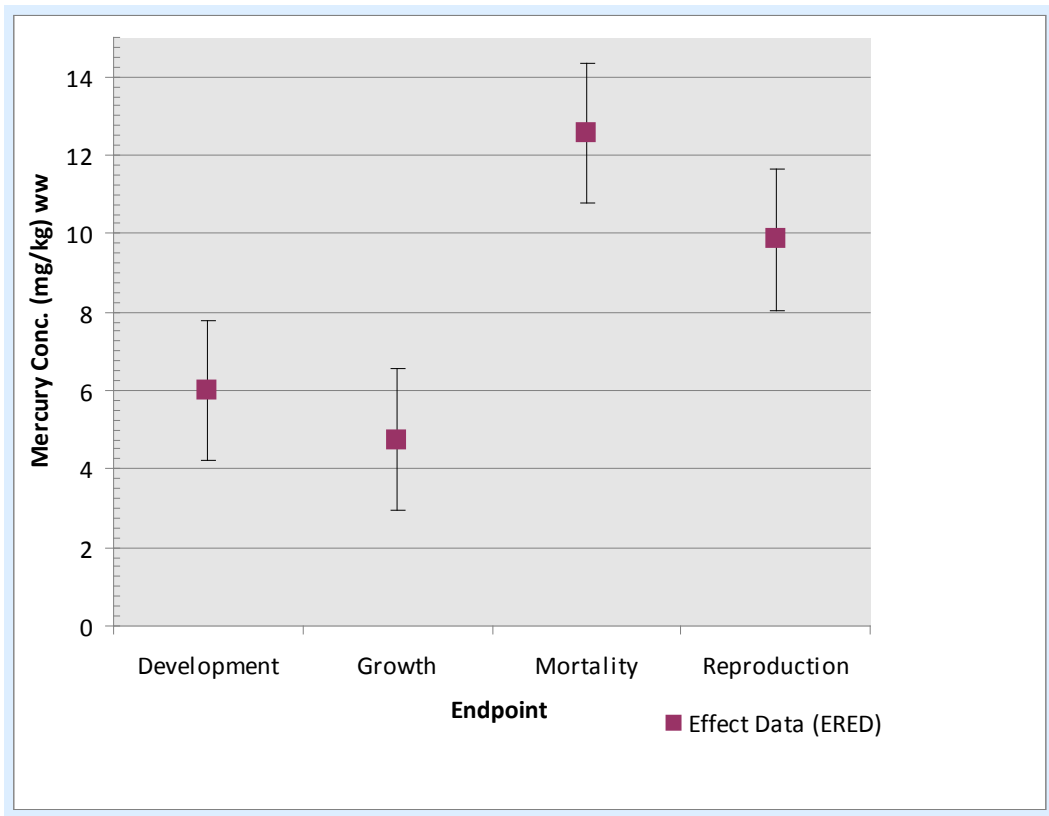


Figure 4-4. Graph from ERED Database Showing Ecological Effects Related to Mercury

5 REFERENCES

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