MEMORANDUM FOR RECORD

SUBJECT: DETERMINATION ON THE SUITABILITY OF PROPOSED DREDGED MATERIAL TESTED FOR THE FORMER SCOTT MILL SITE, ANACORTES, WASHINGTON PROJECT (NWS-2009-203) EVALUATED UNDER SECTION 404 OF THE CLEAN WATER ACT FOR OPEN-WATER DISPOSAL AT THE PORT GARDNER NON-DISPERSIVE DISPOSAL SITE.

1. The following summary reflects the consensus suitability determination of the Agencies that comprise the regional Dredged Material Management Program (DMMP) for the State of Washington on testing conducted for the dredging of the Former Scott Mill Site[1], Anacortes, Washington. The agencies include the Corps of Engineers, Department of Ecology, Department of Natural Resources, and the Environmental Protection Agency. The agencies are charged with determining the suitability of the proposed 25,640 cy of proposed dredged material from the Former Scott Mill Site, located in Anacortes, for open-water disposal at the Port Gardner disposal site.

2. **Table 1** documents the regulatory tracking information and dates for the DMMP testing conducted.

Table 1. Regulatory Tracking Information and Dates

SAP submittal date:	April 28, 2009
SAP approval date: Note: The applicant's AE initiated field sampling prior to DMMP SAP approval due to expedited schedule. The DMMP agencies provided approval recommendations to applicant's AE by phone and email	May 19, 2009
Sampling date(s):	May 18-19, 2009
Characterization Report submittal: Final Biological Testing Report submittal:	June 15, 2009 June 19, 2009
Volume Tested (# DMMUs), Sampling Method:	25,640 cy; (7 DMMUs); Vibracorer
DAIS Tracking Number:	SPMAN-1-B-F-273
Recency Determination Date(s): High = 2 years	May 2011 (**High**)

Background:

3. The project is located in a MTCA cleanup and habitat restoration area, at the Former Scott Paper Company Mill Site in Anacortes, Washington. The cleanup/restoration project is being accomplished under a Consent Decree with the implementing parties (Kimberly Clark) and the Department of Ecology, as part of the "Puget Sound Initiative".

4. A portion of the proposed project was excluded from the DMMP characterization, after DMMP agencies reviewed the MTCA RI data, which noted biological testing exceedance of the CSL (Cleanup Screening Level) in proposed Dredged Material Management Unit-1 (DMMU-1). Six of the remaining seven DMMUs exhibited CSL exceedances for Total Volatile Solids (TVS) and/or wood waste debris, but no CSL level exceedances for other chemicals of concern or biological testing CSL exceedances. One DMMU (DMMU-2) also exhibited a CSL exceedance for 4-Methylphenol, quantitated at 680 ppb, which is above the DMMP screening level of 670 ppb, but well below the DMMP maximum level of 3,600 ppb). The DMMP agencies concluded that DMMP testing of these seven DMMUs would be allowed, consistent with the existing program policy requirements for testing wood waste (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/dmmo/arm_wood.pdf).

[1] Scott Paper Company merged with Kimberly-Clark in 1995.

5. The initial proposed volume of 29,670 cy was reduced by 4,030 cy, reflecting the removal of DMMU-1 from testing for the reasons noted above, which made the effective volume 25,640 cy for DMMP characterization.The DMMP requires biological testing of all material with TVS greater than 25% by weight. The applicant elected to conduct concurrent chemical and toxicity testing.

6. The project was ranked High for DMMP characterization. A SAP was prepared and submitted to DMMP for review on April 28, 2009. The DMMP agencies asked for additional background data relative to dioxin concentrations surrounding and within the proposed dredging/cleanup area.

7. After completing this review, the DMMP agencies concluded that the DMMP characterization of this material would be allowed, and stipulated a 3 ft vertical testing horizon from the mud-water interface, with the first 2 ft evaluated for potential dredging and open-water disposal at the Port Gardner non-dispersive site, and the 2-3 ft vertical horizon designated as the Z-layer, to be archived, pending DMMP review of analysis results.

Sampling:

8. **Figure 1** depicts the site vicinity and **Figure 2** depicts the proposed station locations within each of the designated DMMU boundaries. Two vibracore samples were collected within each of the seven DMMUs on May 18-19, 2009, and composited for seven DMMU analyses. The Data Characterization Report was submitted to the DMMP agencies for review on June 15, 2009. The DMMP agencies concluded that the data was acceptable for decision-making using best-professional-judgment.

Chemical Testing Results:

9. The conventional and chemical analyses results are summarized in **Table 2**. It demonstrates that no chemicals exceeded DMMP screening level guidelines or bioaccumulation triggers with the exception of dioxins/furans (see paragraph 10). However, Total Volatile Solids (TVS) were quantitated at or exceeded the DMMP wood waste threshold of 25% by weight in DMMU-4, DMMU-5, and DMMU-8. The remaining DMMUs were under the TVS threshold. All seven DMMUs underwent concurrent DMMP toxicity testing, and the results of those analyses are described below in paragraph 13.

10. **Table 3** provides a summary of the validated dioxin/furan congener specific testing results for the seven DMMUs, with TEQ (Toxicity Equivalence) concentrations ranging from a low of 0.31 pptr (DMMU-6) to a high of 11.9 pptr (DMMU-4). Except for DMMU-4, the remaining DMMUs depicted relatively low dioxin concentrations up to 5.0 in DMMU-2 (average of 5.0 and 4.9 pptr-TEQ). The volume weighted average summary of the dioxin/furan data are provided in **Table 4**, and discussed in paragraph 12. Based on these chemical and biological testing results, the DMMP agencies concluded that analyses of archived Z-samples underlying each of the six DMMUs meeting the DMMP open-water disposal guidelines would not be required. The DMMP agencies will require Z-sample analysis for DMMU-4 to verify compliance with the Washington State antidegrdation policy, if post-dredge capping is not in the plans for the project. The dredging and upland disposal of DMMU-1, which was not evaluated further in this suitability determination will be accomplished under Ecology TCP oversight and direction.

Dioxin Interim Interpretative Framework:

11. The DMMP agencies are currently using an interim process for interpreting dioxin data (http://www.nws.usace.army.mil/PublicMenu/Menu.cfm?sitename=DMMO&pagename=Dioxin_Guidelines) pending the development of a programmatic regulatory framework, expected in late 2009. The interim guidelines provide a project specific comparison of dioxin/furan concentrations in project dredged material to the disposal site background outside the disposal site. The guidelines applicable to the Port Gardner non-dispersive disposal site specify the following:

- Comparison of dioxin in test sediments to disposal-site background
- Background is defined using disposal site specific monitoring, which defined an offsite maximum concentration of 5.2 pptr-TEQ, and an offsite average concentration of 4.1 pptr-TEQ
- Dioxin concentrations in any given DMMU may not exceed the site maximum (5.2 pptr-TEQ)
- Average dioxin concentrations (weighted to the volume of each DMMU cannot exceed the mean site concentration (4.1 pptr-TEQ)

Dioxin Interpretation on Suitability for Unconfined-Open-Water Disposal:

12. As summarized in paragraph 10 above, six of the seven DMMUs were quantitated below the site maximum of 5.2 pptr-TEQ. DMMU-4 was quantitated at 11.9 pptr-TEQ, which exceeds the Port Gardner offsite maximum concentration of 5.2 pptr-TEQ, and is not suitable for disposal at the Port Gardner site under the interim guidelines. **Table 4** provides the volume weighted averages for all DMMU's. The volume weighted average concentration for the six DMMUs below 5.2 pptr-TEQ (cumulative total volume of 21,660 cy) is 2.76 pptr-TEQ, which is below the interim Port Gardner offsite average of 4.1 pptr-TEQ. Therefore, these six DMMUs are suitable for open-water disposal at the Port Gardner non-dispersive site under the interim Dioxin Guidelines.

Biological Testing Summary:

13. Toxicity testing was initiated concurrently with the chemical analysis of sediment samples by NewFields Northwest. Interpretative Guidelines for each test within the DMMP testing suite are depicted in **Table 5.** The tests included the 10-day Amphipod mortality test (*Eohaustorius estuarius*), ~48-hour Sediment Larval Test (*Dendraster excentricus*), and 20-day juvenile polychaete growth bioassay (*Neanthes arenaceodenta*).

14. Because of concerns about how the Sediment Larval Test might perform in sediments with a relatively large amount of wood waste, the DMMP agencies reviewed and approved the use of a side-by-side comparison using a slight protocol variation of the Puget Sound Estuary Program (PSEP) sediment larval protocol, which utilizes a screen tube (25 um mesh) within each test beaker as described in **Attachment 1**. For the amended Sediment Larval Protocol, the DMMP agencies were concerned about achieving a statistical Power of $\geq$0.80 in discriminating between the test results and reference sediment, and NewFields recommended increasing the within test replication requirement from 5 to 7 for this protocol to achieve that objective. The DMMP agencies agreed using Best-Professional-Judgment (BPJ) to increase the within sample replication requirement.

15. Reference samples were collected in Carr Inlet, and the test sediments were matched with appropriate reference sediments to evaluate the grain-size effect on bioassays. Due to the influence of woody debris on grain size results, the test sediments and reference samples were subjected to grain size analysis on an as-received bases and an organic-free basis after removal of organic matter after processing in a muffle furnace (440 ^{o}C) as depicted in **Table 2**. The samples from DMMUs 2-8 exhibited 43 to 81% fines in the standard as-received evaluation, as compared to 55 to 89% in the organic-free samples. All of the samples were more closely matched with Carr Reference (CR-1) which contained 80 to 81% fines in both the standard and organic-free grain size determinations.

16. The control and reference sediments generally met the DMMP performance requirements for all three bioassays, except the CR-1 reference did not meet the DMMP performance standard for the *Neanthes* Bioassay; thus, for the *Neanthes* bioassay, all treatments were compared to CR-22 reference instead of the CR-1 reference. Testing results are summarized for each bioassay in **Table 6**, and below.

    - **Amphipod Bioassay**. Test results for the seven DMMUs compared to Carr Inlet Reference (CR-1) indicated that DMMU-7 exhibited a 2-Hit response relative to DMMP Non-Dispersive Interpretative

Guidelines (**Tables 5** and **6**), whereas the other six DMMUs passed the interpretative guidelines for non-dispersive sites.

- **Neanthes-20-day Growth Bioassay**. The CR-1 reference sample did not meet the performance standard for this bioassay, and all test sediments were compared to Carr Reference Station CR-22. All seven DMMUs passed the interpretative guidelines for non-dispersive sites, and no hits were recorded.

- **Echinoderm (Sand-Dollar)-Sediment Larval Bioassay.** All seven DMMUs utilizing the standard PSEP protocol passed the interpretative guidelines for non-dispersive sites, and no hits were recorded. The data collected for the screen-tube variation of the PSEP protocol were similar to those from the standard assay (all DMMUs passed non-dispersive site guidance) but are not being used for decision-making for this suitability determination. The modified bioassay results will be used by the DMMP agencies to evaluate the potential use of this protocol for future DMMP projects in wood waste areas.

Suitability Determination:

17. The results of these analyses in summary indicate that 21,660 cy of proposed dredged material are suitable for unconfined-open-water disposal at the Port Gardner non-dispersive site, and 3,980 cy represented by DMMU-4 are unsuitable for unconfined-open-water disposal, and must be disposed at an Ecology approved upland disposal site. The 4,030 cy of material in DMMU-1 not evaluated by DMMP, but evaluated as exceeding CSL criteria in the MTCA RI is not suitable for open-water disposal, and will be dredged and disposed at an Ecology approved upland disposal site.

18. This memorandum documents the suitability of material proposed for dredging from the former Scott Paper Mill site in Anacortes, Washington, for open-water disposal at the Port Gardner disposal site. However, this suitability determination does not constitute final agency approval of the project. A dredging plan for this project must be completed as part of the final project approval process. A final decision will be made after full consideration of agency input, and after an alternatives analysis is done under Section 404(b)(1) of the Clean Water Act.

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Concur:

24 June 2009
Date — David Kendall, Ph.D., Seattle District Corps of Engineers

June 24, 2009
Date — Laura Inouye, Ph.D. Washington Department of Ecology

6/24/09
Date — Erika Hoffman, Environmental Protection Agency, Region 10

24-June-09
Date — Courtney Wasson, Washington Department of Natural Resources

Randel Perry, Corps Regulatory PM
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DMMO File

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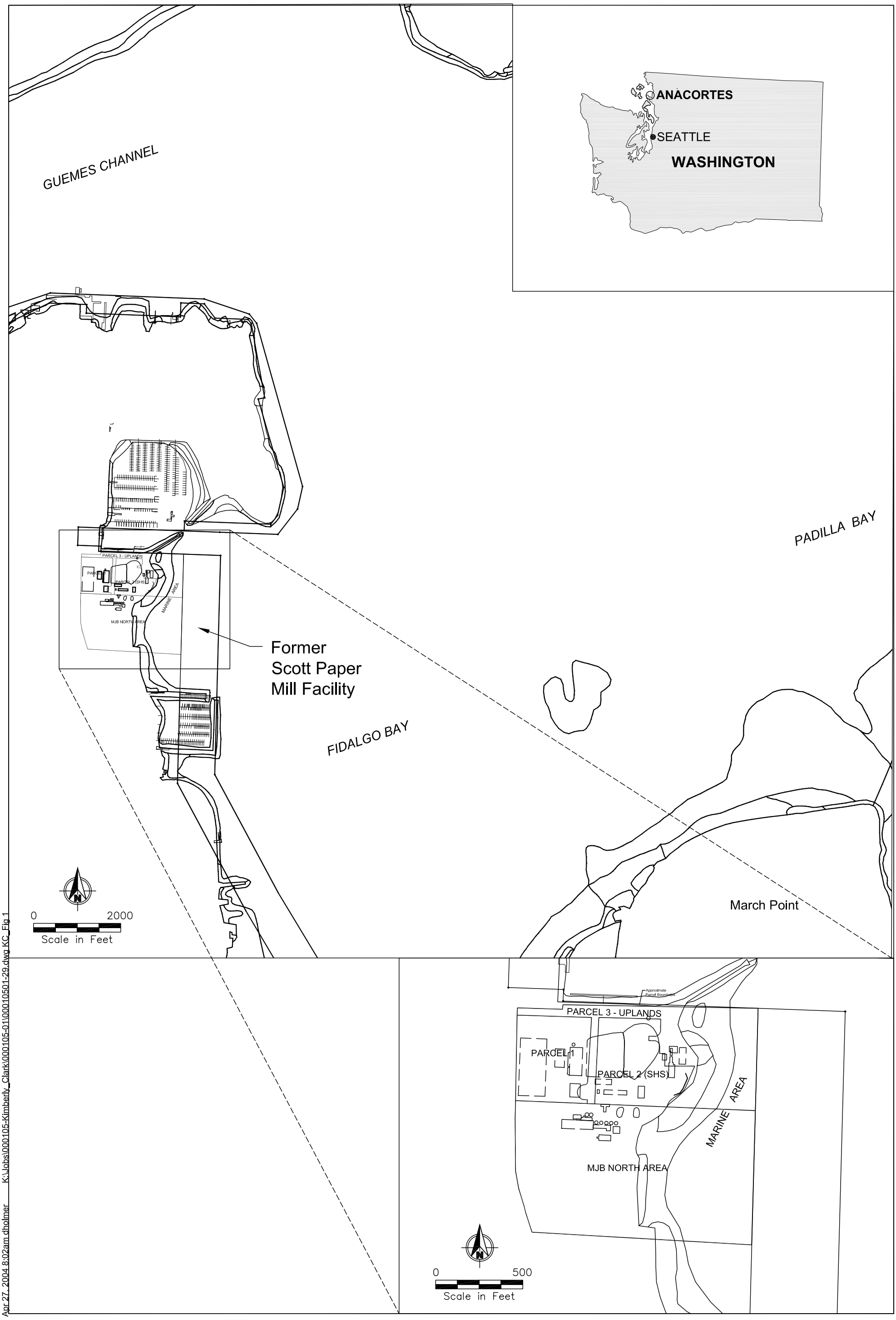

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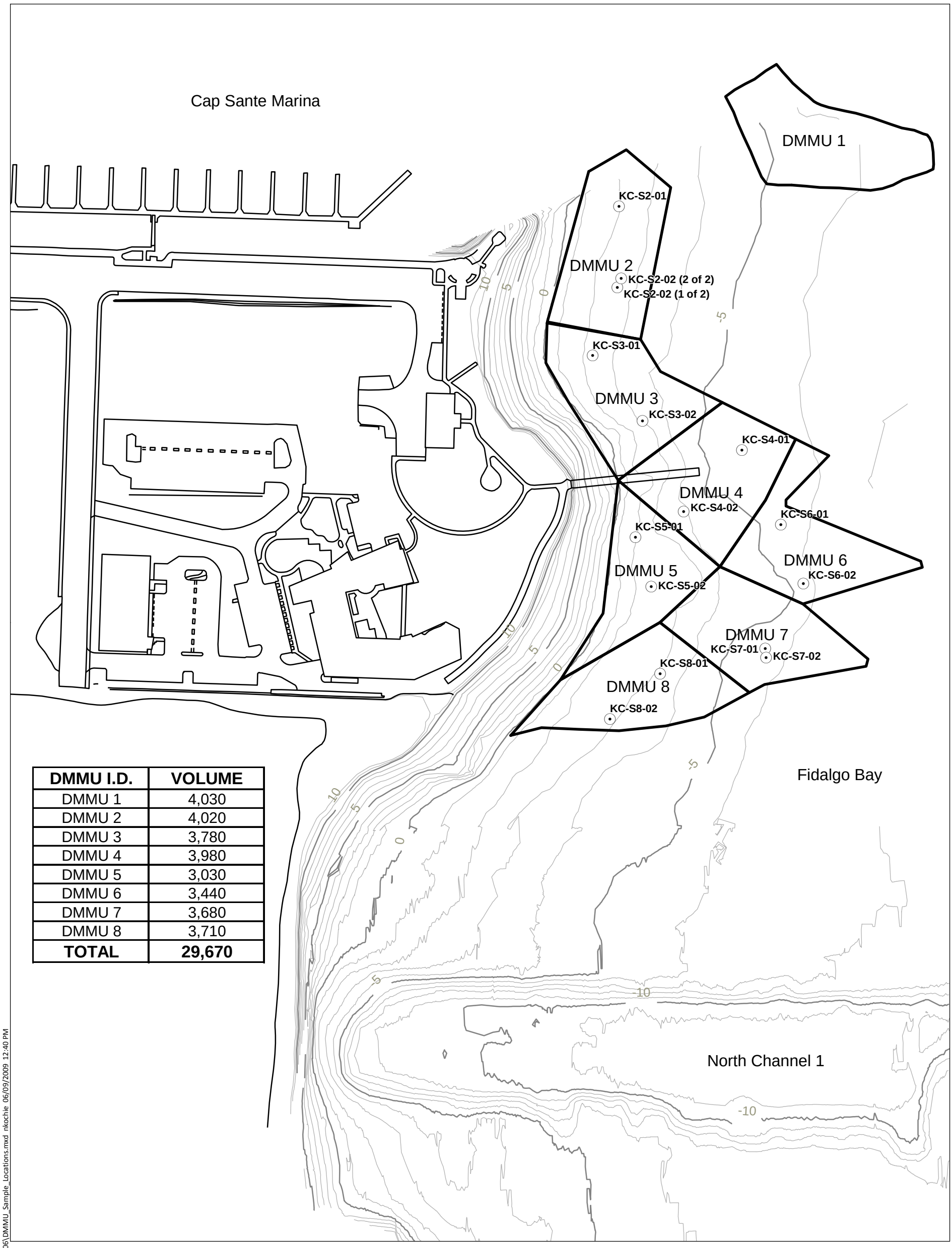
Figure 1
Vicinity Map
FORMER SCOTT PAPER MILL SITE



DMMU I.D.	VOLUME
DMMU 1	4,030
DMMU 2	4,020
DMMU 3	3,780
DMMU 4	3,980
DMMU 5	3,030
DMMU 6	3,440
DMMU 7	3,680
DMMU 8	3,710
TOTAL	**29,670**

Actual Sample Locations and I.D.

DMMU

1-foot Bathymetric Contours - Elevation in Feet (MLLW)

Feet

0 150 300

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Figure 2
DMMU Sampling Locations
Former Scott Paper Mill

Table 2. DMMP Testing Summary for Former Scott Paper Mill Site

DMMU ID: Sample ID: Sample Date: Depth: Sample Type:	DMMP SL Marine Guideline	DMMP BT	DMMP ML Marine Guideline	DMMU-2 KC-S2-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-2 KC-S52-CS-090520 5/20/2009 0 -2 ft Field Duplicate	DMMU-3 KC-S3-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-4 KC-S4-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-5 KC-S5-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-6 KC-S6-CS-090519 5/19/2009 0 -2 ft Normal	DMMU-7 KC-S7-CS-090519 5/19/2009 0 -2 ft Normal	DMMU-8 KC-S8-CS-090519 5/19/2009 0 -2 ft Normal	Bioassay Ref. CR-1 6/4/2009 Normal	Bioassay Ref. CR-22 6/4/2009 Normal
Conventional Parameters (pct)													
Total Solids				42	41	46	45	37	66	63	46	56	75
Total Volatile Solids	25%			21	19	24	25 †	33 †	3.2	3.6	33 †	2.6	0.6
Ash Content				79	81	76	75	67	97	96	67		
Total Organic Carbon				5.4	5.8	4.7	3.9	6.6	0.81	0.9	3.9		
Ammonia (mg/kg)				73	70	27	59	23	12	12	17		
Sulfide (mg/kg)				130	280	410	630	370	52 J	17 J	340 J		
Grain Size (pct)													
Total Gravel				16	--	5.6	1.1	9.9	0.3	4.6	21	0	1.9
Total Sand				18	--	27	18	43	22	21	36	20	91
Total Silt				44	--	49	60	33	63	61	31	69	5.4
Total Clay				22	--	19	21	14	15	14	12	11	1.5
Total Fines (silt + clay)				66	--	68	81	47	78	75	43	80	7
Muffled Grain Size (pct)													
Total Gravel				0.1	--	2.9	0.2	2.3	0.9	0.1	0.2	0	0.4
Total Sand				11	--	31	16	43	19	25	35	19	94
Total Silt				81	--	60	74	45	72	65	55	76	4.7
Total Clay				8.4	--	7	10	9.3	8.8	9.7	10	5.5	1
Total Fines (silt + clay)				89	--	67	84	55	81	75	65	81	5.7
Metals (mg/kg)													
Antimony	150		200	10 UJ	10 UJ	10 UJ	10 UJ	10 UJ	7 UJ	7 UJ	10 UJ		
Arsenic	57	507.1	700	10 U	10 U	10 U	10 U	10 U	15	14	10		
Cadmium	5.1	11.3	14	1.6	0.7	1.2	1.5	0.9	0.9	0.9	1.6		
Chromium		267		32	14	28	32	23	37	36	32		
Copper	390	1027	1300	28	13	22	42	60	21	16	59		
Lead	450	975	1200	14	6	11	22	12	3	3	36		
Mercury	0.41	1.5	2.3	0.1	0.09	0.05	0.13	0.06 U	0.03	0.03 U	0.06		
Nickel	140	370	370	25	11	21	24	15	26	26	25		
Selenium		3		1 U	1 U	1 U	1 U	1 U	0.7 U	0.7 U	1 U		
Silver	6.1	6.1	8.4	0.6 U	0.6 U	0.6 U	0.6 U	0.8 U	0.4 U	0.4 U	0.7 U		
Zinc	410	2783	3800	77	29	58	73	37	55	48	68		
Organometallic compounds (µg/L)													
Tributyltin (ion) (PW)	0.15	0.15		0.008 U	0.008 U	0.008 U	0.008 U	0.008 UJ	0.008 U	0.008 U	0.008 U		
Dibutyltin (ion) (PW)				0.012 UJ	0.012 UJ	0.012 UJ	0.012 UJ	0.012 U	0.012 UJ	0.012 UJ	0.012 UJ		
Butyltin (ion) (PW)				0.02 J	0.008 UJ	0.008 UJ	0.021 J	0.008 UJ	0.008 U	0.008 U	0.019		
PCB Aroclors (µg/kg)													
Aroclor 1016				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Aroclor 1221				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Aroclor 1232				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Aroclor 1242				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Aroclor 1248				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Aroclor 1254				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Aroclor 1260				9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
Total PCB (U = 0)	130		3100	9.9 U	9.9 U	9.9 U	9.7 U	9.9 U	9.6 U	9.8 U	9.8 U		
PCB Aroclors (mg/kg-OC)													
Aroclor 1016				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aroclor 1221				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aroclor 1232				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aroclor 1242				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aroclor 1248				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aroclor 1254				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aroclor 1260				0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		

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Total PCB (U = 0)		38		0.18 U	0.17 U	0.21 U	0.25 U	0.15 U	1.2 U	1.1 U	0.2513 U		
Aromatic Hydrocarbons (µg/kg)													
Total LPAH (U = 0)	5200		29000	**140**	**160**	**64**	**68**	**70**	**58**	19 U	**120**		
Naphthalene	2100		2400	**24**	**44**	20 U	20 U	20 U	19 U	19 U	**15 J**		
Acenaphthylene	560		1300	20 U	**11 J**	20 U	20 U	20 U	19 U	19 U	19 U		
Acenaphthene	500		2000	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Fluorene	540		3600	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Phenanthrene	1500		21000	**73**	**71**	**14 J**	**18 J**	**20 J**	**10 J**	19 U	**61**		
Anthracene	960		13000	**10 J**	**11 J**	20 U	20 U	20 U	19 U	19 U	**11 J**		
2-Methylnaphthalene	670		1900	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Total HPAH (U = 0)	12000		69000	**500**	**520**	**140**	**160**	**160**	**95**	19 U	**350**		
Fluoranthene	1700	4600	30000	**96**	**120**	**22**	**26**	**26**	19 U	19 U	**86**		
Pyrene	2600	11980	16000	**130**	**140**	**33**	**47**	**36**	**9.9 J**	19 U	**69**		
Benzo(a)anthracene	1300		5100	**36**	**33**	**12 J**	**10 J**	**12 J**	19 U	19 U	**31**		
Chrysene	1400		21000	**58**	**50**	**13 J**	**14 J**	**16 J**	19 U	19 U	**36**		
Benzo(b)fluoranthene				**31**	**26**	20 U	20 U	**11 J**	19 U	19 U	**23**		
Benzo(k)fluoranthene				**40**	**38**	**11 J**	20 U	20 U	19 U	19 U	**23**		
Total Benzofluoranthenes (b,j,k) (U = 0)	3200		9900	**71**	**64**	**21**	20 U	**21**	19 U	19 U	**46**		
Benzo(a)pyrene	1600		3600	**46**	**45**	20 U	**12 J**	**13 J**	19 U	19 U	**34**		
Indeno(1,2,3-c,d)pyrene	600		4400	**20**	**22**	20 U	20 U	20 U	19 U	19 U	**16 J**		
Dibenzo(a,h)anthracene	230		1900	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Benzo(g,h,i)perylene	670		3200	**28**	**32**	20 U	20 U	**11 J**	19 U	19 U	**23**		
Total PAH (U = 0)				**630**	**670**	**210**	**230**	**230**	**150**	19 U	**470**		
1-Methylnaphthalene				20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Chlorinated Hydrocarbons (µg/kg)													
1,3-Dichlorobenzene	170			1.8 U	1.9 U	1.7 U	1.7 UJ	2.1 U	1.2 U	1.2 U	1.9 UJ		
1,4-Dichlorobenzene	110		120	1.8 U	1.9 U	1.7 U	1.7 UJ	2.1 U	1.2 U	1.2 U	1.9 UJ		
1,2-Dichlorobenzene	35		110	1.8 U	1.9 U	1.7 U	1.7 UJ	2.1 U	1.2 U	1.2 U	1.9 UJ		
1,2,4-Trichlorobenzene	31		64	8.8 U	9.4 U	8.3 U	8.6 UJ	11 U	5.9 U	5.9 U	9.3 UJ		
Hexachlorobenzene	22	168	230	0.99 U	0.99 U	0.99 U	0.98 U	0.98 U	0.96 U	0.98 U	0.98 U		
Phthalates (µg/kg)													
Dimethyl phthalate	71		1400	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Diethyl phthalate	200		1200	**17 J**	**18 J**	**18 J**	20 U	20 U	19 U	19 U	19 U		
Di-n-butyl phthalate	1400		5100	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Butylbenzyl phthalate	63		970	20 U	20 U	20 U	20 U	**18 J**	19 U	19 U	19 U		
Bis(2-ethylhexyl) phthalate	1300		8300	20 U	20 U	20 U	20 U	20 U	58 U	40 U	49 U		
Di-n-octyl phthalate	6200		6200	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Phenols (µg/kg)													
Phenol	420		1200	**62**	20 U	**31**	**45**	**73**	**29**	**23**	**40**		
2-Methylphenol (o-Cresol)	63		77	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
4-Methylphenol (p-Cresol)	670		3600	**44**	**54**	**16 J**	**38**	20 U	19 U	19 U	**60**		
2,4-Dimethylphenol	29		210	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Pentachlorophenol	400	504	690	99 U	98 U	100 U	98 U	98 U	97 U	96 U	97 U		
Miscellaneous Extractables (µg/kg)													
Benzyl alcohol	57		870	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Benzoic acid	650		760	200 U	200 U	200 UJ	200 U	200 U	190 UJ	190 UJ	190 UJ		
Dibenzofuran	540		1700	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Hexachloroethane	1400		14000	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Hexachlorobutadiene	29		270	0.99 U	0.99 U	0.99 U	0.98 U	0.98 U	0.96 U	0.98 U	0.98 U		
N-Nitrosodiphenylamine	28		130	20 U	20 U	20 U	20 U	20 U	19 U	19 U	19 U		
Volatile Organics (µg/kg)													
Ethylbenzene	10		50	1.8 U	1.9 U	1.7 U	1.7 U	2.1 U	1.2 U	1.2 U	1.9 U		

Table 2. DMMP Testing Summary for Former Scott Paper Mill Site

DMMU ID: Sample ID: Sample Date: Depth: Sample Type:	DMMP SL Marine Guideline	DMMP BT	DMMP ML Marine Guideline	DMMU-2 KC-S2-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-2 KC-S52-CS-090520 5/20/2009 0 -2 ft Field Duplicate	DMMU-3 KC-S3-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-4 KC-S4-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-5 KC-S5-CS-090520 5/20/2009 0 -2 ft Normal	DMMU-6 KC-S6-CS-090519 5/19/2009 0 -2 ft Normal	DMMU-7 KC-S7-CS-090519 5/19/2009 0 -2 ft Normal	DMMU-8 KC-S8-CS-090519 5/19/2009 0 -2 ft Normal	Bioassay Ref. CR-1 6/4/2009 Normal	Bioassay Ref. CR-22 6/4/2009 Normal
Trichloroethene (TCE)	160		1600	1.8 U	1.9 U	1.7 U	1.7 U	2.1 U	1.2 U	1.2 U	1.9 U		
Tetrachloroethene (PCE)	57		210	1.8 U	1.9 U	1.7 U	1.7 U	2.1 U	1.2 U	1.2 U	1.9 U		
o-Xylene				1.8 U	1.9 U	1.7 U	1.7 U	2.1 U	1.2 U	1.2 U	1.9 U		
m,p-Xylene				1.8 U	1.9 U	1.7 U	1.7 U	2.1 U	1.2 U	1.2 U	1.9 U		
Total Xylene (U = 0)	40		160	1.8 U	1.9 U	1.7 U	1.7 U	2.1 U	1.2 U	1.2 U	1.9 U		
Pesticides & PCBs (µg/kg)													
4,4'-DDD (p,p'-DDD)				2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
4,4'-DDE (p,p'-DDE)				2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
4,4'-DDT (p,p'-DDT)				2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
Total DDT (U = 0)	6.9	50	69	2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
alpha-Chlordane (cis-Chlordane)				1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U		
gamma-Chlordane				1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U		
Total Chlordane (U = 0)	10	37		2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
Aldrin	10			1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U		
cis-Nonachlor				2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
Dieldrin	10			2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
gamma-BHC (Lindane)	10			1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.98 U		
Heptachlor	10			1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.98 U		
Oxychlordane				2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
trans-Nonachlor				2 U	2 U	2 U	2 U	2 U	1.9 U	2 U	2 U		
Dioxin/Furans: ng/kg-TEQ (U = 1/2 DL) (See Table 3)	5.2 (PG)			5	4.9	4	**11.9†**	3.5	0.31	0.47	2.9		
Biological Testing: (NH = Non Hit; 2H = 2-HIT; 1H = 1-Hit)													
Eohaustorius estuarius hits:				NH		NH	NH	NH	NH	**2H**	NH		
Dendraster exentricus hits:				NH		NH	NH	NH	NH	NH	NH		
Neanthes arenaceodentata hits:				NH		NH	NH	NH	NH	NH	NH		
Bioassay Determination: (P/F Non-dispersive Guidelines)				**PASS**		**PASS**	PASS	PASS	PASS	PASS	**PASS**		
BTs exceeded: (yes/no)				no		no	no	no	no	no	no		
Bioaccumulation Conducted: (yes/no)				no		no	no	no	no	no	no		
ML Rule ExceededDMMP Determination (yes/no)				no		no	no	no	no	no	no		
Mudline Elevation (ft, MLLW)				-2.6, -2.6	-2.5	-2.3, -3.1	-6.3, -3.7	-1.5, -2	-5.2, -5.3	-5.4, -5.4	-3.6, -2.9		
DMMU Volume (cubic yards):				4,020		3,780	3,980	3,030	3,440	3,680	3,710		
DMMP Determination: (Suitable / Unsuitable UCOWD):				**SUITABLE**	**SUITABLE**	**SUITABLE**	**UNSUITABLE**	**SUITABLE**	**SUITABLE**	**SUITABLE**	**SUITABLE**		

Legend:

† Detected concentration exceeds one or more DMMP Guidelines

2-Hit Response

PASS (Non-Dispersive Site Toxicity Guidelines)

SUITABLE (NON-DISPERSIVE SITE)

UNSUITABLE (NON-DISPERSIVE SITE)

Bold = Detected result

J = Estimated value

U = Compound analyzed, but not detected above detection limit

UJ = compound analyzed, bt not detected above estimated detection limit

Benzo(j)fluoranthene is included in the total of benzo(b&k)fluoranthenes

Sum DDT consists of the sum of 4,4'□DDD, 4,4'□DDE, and 4,4'□DDT

Total Chlordane includes alpha-chlordane (cis-chlordane), beta-chlordane (trans-chlordane), cis-nonaclor, trans-nonaclor and oxychlordane

Total xylene is the sum of o-,m-,p-isomers

Totals are calculated as the sum of all detected results (U=0). If all results are not detected, the highest reporting limit is reported as sum.

U=1/2; Half of the detection limit is included in the sum of results (applies to dioxin/furan TEQ calculation only)

Results not reported or not applicable

mg/kg = milligrams per kilogram

ug/kg = micrograms per kilogram

ng/kg = nanograms per kilogram

µg/L = micrograms per liter

PW = porewate

N = Normal Field Sample

FD = Field Duplicate

Toxicity Equivalency (TEQ) values

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Table 3. Summary of Dioxin/Furan Data from the Former Scott Paper Mill DMMP Characterization

DMMU ID: Sample ID: Sample Date: Depth: Sample Type:	WHO (05)	DMMU-2 KC-S2-CS-090520 5/20/2009 0 -2 ft Normal			DMMU-2 (dup) KC-S52-CS-090520 5/20/2009 0 -2 ft Field Duplicate			3 KC-S3-CS-090520 5/20/2009 0 -2 ft Normal			DMMU-4 KC-S4-CS-090520 5/20/2009 0 -2 ft Normal			DMMU-5 KC-S5-CS-090520 5/20/2009 0 -2 ft Normal			DMMU-6 KC-S6-CS-090519 5/19/2009 0 -2 ft Normal		
Dioxin Furans (ng/kg)	**TEF**	ng/kg-dw	LQ	TEQ	ng/kg-dw	LQ	TEQ	ng/kg-dw	LQ	TEQ	ng/kg-dw	LQ	TEQ	ng/kg-dw	LQ	TEQ	ng/kg-dw	LQ	TEQ
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	1	0.85		0.85	0.84		0.84	0.79	j	0.79	1		1	0.61		0.61	0.1	U	0.05
1,2,3,7,8-Pentachlorodibenzo-p-dioxin (PeCDD)	1	1.9	j	1.9	1.9	j	1.9	1.6	j	1.6	2.5	j	2.5	1.2	j	1.2	0.1	U	0.05
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin (HxCDD)	0.1	1.4	j	0.14	1.1	j	0.11	1.4	j	0.14	2.3	j	0.23	0.9	j	0.09	0.3	U	0.015
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin (HxCDD)	0.1	3		0.3	2.6	j	0.26	2.6		0.26	13		1.3	2.3	j	0.23	0.22	j	0.022
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin (HxCDD)	0.1	1.9	j	0.19	1.8	j	0.18	2.1	j	0.21	4.2		0.42	1.3	j	0.13	0.18	j	0.018
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD)	0.01	24		0.24	19		0.19	9.1		0.091	170		1.7	14		0.14	1.9	j	0.019
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (OCDD)	0.0003	71		0.0213	68		0.0204	19		0.0057	1600		0.48	67		0.0201	10		0.003
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	0.1	3.5		0.35	3.6		0.36	3		0.3	3.6		0.36	2.4		0.24	0.19	j	0.019
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	0.03	1.8	j	0.054	1.8	j	0.054	1.4	j	0.042	2	j	0.06	1.3	j	0.039	0.07	U	0.00105
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	0.3	2.2	j	0.66	2.2	j	0.66	1.3	j	0.39	4.5		1.35	1.5	j	0.45	0.07	U	0.0105
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	0.1	0.98	j	0.098	0.9	j	0.09	0.61	j	0.061	4.1		0.41	0.7	j	0.07	0.47	U	0.0235
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	0.1	0.95	j	0.095	0.89	j	0.089	0.61	j	0.061	2.5		0.25	0.91	j	0.091	0.5	U	0.025
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	0.1	0.17	j	0.017	0.24	j	0.024	0.19	j	0.019	0.94	j	0.094	0.33	U	0.0165	0.61	U	0.0305
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	0.1	0.88	j	0.088	0.83	j	0.083	0.67	j	0.067	4		0.4	0.92	j	0.092	0.48	U	0.024
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	0.01	4.2		0.042	4.2		0.042	1.2	j	0.012	120		1.2	3.7		0.037	0.34	j	0.0034
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	0.01	0.29	j	0.0029	0.3	j	0.003	0.17	U	0.00085	4.6		0.046	0.23	U	0.00115	0.13	U	0.00065
1,2,3,4,5,6,7,8-Octachlorodibenzofuran (OCDF)	0.0003	4.4	j	0.00132	5.1		0.00153	0.935	j	0.0002805	220		0.066	3.5	j	0.00105	0.64	j	0.000192
Total TEQ (u = 1/2)(Mammal):				5.0			4.9			4.0			11.9			3.5			0.31
Total TEQ (u=0)(Mammal):				5.0			4.9			4.0			11.9			3.4			0.085
Total Organic Carbon (TOC), %:				5.4			5.8			4.7			3.9			6.6			0.81

Table 3. Summary of Dioxin/Furan Data from the Former Scott Paper Mill DMMP Characterization

DMMU ID: Sample ID: Sample Date: Depth: Sample Type:	WHO (05)	DMMU-7 KC-S7-CS-090519 5/19/2009 0 -2 ft Normal			DMMU-8 KC-S8-CS-090519 5/19/2009 0 -2 ft Normal		
Dioxin Furans (ng/kg)	**TEF**	ng/kg-dw	LQ	TEQ	ng/kg-dw	LQ	TEQ
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	1	**0.11**	**j**	**0.11**	**0.66**	**j**	**0.66**
1,2,3,7,8-Pentachlorodibenzo-p-dioxin (PeCDD)	1	**0.1**	**j**	**0.1**	**1.22**	**j**	**1.22**
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin (HxCDD)	0.1	0.3	U	0.015	**0.67**	j	**0.067**
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin (HxCDD)	0.1	0.3	U	0.015	**1.2**	j	**0.12**
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin (HxCDD)	0.1	0.32	U	0.016	**0.91**	j	**0.091**
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD)	0.01	**1.4**	**j**	**0.014**	**5.4**	**j**	**0.054**
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (OCDD)	0.0003	**9.6**		**0.00288**	**13**		**0.0039**
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	0.1	**0.82**		**0.082**	**2.1**		**0.21**
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	0.03	**0.34**	**j**	**0.0102**	**1.1**	**j**	**0.033**
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	0.3	**0.2**	**j**	**0.06**	**1.2**	**j**	**0.36**
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	0.1	**0.092**	**j**	**0.0092**	**0.53**	**j**	**0.053**
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	0.1	**0.062**	**j**	**0.0062**	**0.53**	**j**	**0.053**
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	0.1	0.15	U	0.0075	0.084	U	0.0042
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	0.1	0.11	U	**0.0055**	**0.46**	j	**0.046**
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	0.01	**0.17**	**j**	**0.0017**	**0.92**	**j**	**0.0092**
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	0.01	0.17	U	0.00085	0.1	U	0.0005
1,2,3,4,5,6,7,8-Octachlorodibenzofuran (OCDF)	0.0003	0.63	U	9.4500E-05	**0.95**	j	**0.000285**
Total TEQ (u = 1/2)(Mammal):				**0.46**			**3.0**
Total TEQ (u=0)(Mammal):				**0.41**			**2.7**
Total Organic Carbon (TOC), %:				**0.9**			**3.9**

Table 4. Volume Weighted Average (VWA) Dioxin Concentrations for Former Scott Paper Mill MTCA Dredging Project

DMMU ID:	Depth, ft	Volume (CY)	TCDD/F TEQ	ng/kg-dw	Product (Vol x TEQ)	ng x cy/kg x DMMU	Product/total	Proportional contribution/Suitable DMMU
2	0-2	4,020	5	ng/kg-dw	20,100	ng x cy/kg	33.7%	% of Total DMMU
3	0-2	3,780	4	ng/kg-dw	15,120	ng x cy/kg	25.3%	% of Total DMMU
4*	0-2	3,980	11.9	ng/kg-dw				
5	0-2	3,030	3.5	ng/kg-dw	10,605	ng x cy/kg	17.8%	% of Total DMMU
6	0-2	3,440	0.31	ng/kg-dw	1,066	ng x cy/kg	1.8%	% of Total DMMU
7	0-2	3,680	0.46	ng/kg-dw	1,693	ng x cy/kg	2.8%	% of Total DMMU
8	0-2	3,710	3	ng/kg-dw	11,130	ng x cy/kg	18.6%	% of Total DMMU
Totals (Suitable):		21,660 cy			59,714	ng x cy/kg	2.76	ng/kg-dw/Project (VWA)

*DMMU-4 exceeds the offsite Maximum TCDD/F concentration of 5.2 pptr-TEQ under Interim Guidelines and is unsuitable for Port Gardner Site disposal.

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Table 6. Toxicity Testing Summary for Former Scott Paper Mill

Treatment (Total Fines on Organic-Free Basis)	Amphipod Mortality (*Eohaustorius estuarius*) (Survival, %)	Neanthes 20-Day Growth Bioassay (*Neanthes arenaceodentata*)		Sediment Echindoerm Larval Bioassay[1] (Dendraster excentricus)			DMMP Determination Pass / Fail Non-Dispersive Interpretative Guidelines
		Survival, %	MIG[2] (mg/ind/day)	Normal Survival (%)	N_R/N_C – N_T/N_C	N_T/N_C	
Control	100	100	0.704	96.7	NA	NA	
Reference (CR-1) (Total Fines: 81%)	95	100	0.488**	82.0	NA	NA	
Reference (CR-22) (Total Fines: 5.7%)	NA	96	0.673	NA	NA	NA	
DMMU-2 (Total Fines: 89%)	95 (NS)	100	0.691 (T/R = 1.03)	85.8	-3.8	0.86	PASS
DMMU-3 (Total Fines: 67%)	97 (NS)	100	0.465 (T/R = 0.69)[3]	85.9	-4.0	0.86	PASS
DMMU-4 (Total Fines: 84%)	96 (NS)	100	0.679 (T/R = 1.01)	83.9	-1.9	0.84	PASS
DMMU-5 (Total Fines: 55%)	99 (NS)	96	0.613 (T/R = 0.91)	78.1	3.9	0.78	PASS
DMMU-6 (Total Fines: 81%)	80 (SS)	100	0.743 (T/R = 1.1)	87.6	-5.7	0.88	PASS
DMMU-7 (Total Fines: 75%)	71[2H] (SS)	100	0.639 (T/R = 0.91)	85.8	-3.8	0.86	PASS
DMMU-8 (Total Fines: 65%)	92 (NS)	100	0.517 (T/R = 0.73)	87.0	-5.0	0.87	PASS

* SS = Statistically different than Reference; NS = not statistically different than Reference

**CR-1 did not meet reference performance standard ($MIG_R \div MIG_C \geq 0.80$), and all treatments were compared to CR-22

2H = 2-Hit Response, requires corroborating response to Fail DMMU

[1] Followed standard PSEP Protocol. Screen Tube Protocol results not used in suitability determination.

[2] MIG = mean individual growth rate

[3] MIG_T/MIG_R < 70% but not statistically different, therefore not scored as a hit under 2-Hit Rule.

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1.0 SCOPE

The benthic larval test is part of a suite of test used to evaluate the toxicity of marine sediments in Puget Sound and throughout EPA Region 10. Sediment testing procedures follow those described in **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995), Dinnel and Stober (1995), Standard Methods (APHA 1985), ASTM (1989), and updates provided in the Sediment Management Annual Review Meeting (SMARM).**

2.0 SUMMARY OF TEST

2.1 Approach

Number of samples	≥1
Number of replicates per test sediment	5
Number of controls	≥1
Number of replicates per control	5
Number of reference sediments	≥1
Number of replicates per reference sediment	5
Test chambers	1 L glass beaker or comparable wide mouth glass jar (10 cm internal diameter)
Test sediment volume	18 g of sediment per container
Overlying water	900 mL of 28‰ salinity, clean, filtered, seawater with a maximum holding time of 2 days
Renewal of overlying water	None
Number of test organisms per chamber	20-40 embryos/mL for Bivalves; 20-30 embryos/mL for Echinoderms
Type of biological observations	Survival, Development
Times of biological observations	Post test
Type of physical observations	Room or bath temperature continuous, light daily
Types of water quality analyses	DO, temperature, salinity, pH; ammonia and sulfides (Program Dependent)
Times of water quality samples	DO, temperature, salinity, and pH measured in a surrogate test chamber daily. Ammonia and total sulfide samples taken at the beginning and end of the test.
Aeration	Gentle aeration is applied to all chambers if Dissolved Oxygen levels fall below 6.0 mg/L or high ammonia or sulfides are suspected.
Sediment holding time	Samples must be stored in the dark at 4 ± 2 ° C with no headspace or headspace filled with nitrogen gas.

2.2 Physical Requirements

Species	*Dendraster excentricus*	*Strongylocentrotus purpuratus*	*Mytilus* sp.	*Crassostrea gigas*
DO (mg/L)*	> 4.8	> 4.8	> 4.8	> 4.8
Temperature (°C)	15 ± 1	15 ± 1	16 ± 1	20 ± 1
Salinity (ppt)	28 ± 1	28 ± 1	28 ± 1	28 ± 1
pH	Ambient	Ambient	Ambient	Ambient
Lighting[a]	14 hours light : 10 hours dark at 50-100 foot-candles	14 hours light : 10 hours dark at 50-100 foot-candles	14 hours light : 10 hours dark at 50-100 foot-candles	14 hours light : 10 hours dark at 50-100 foot-candles
Ammonia	< 0.14 mg/L unionized	< 0.14 mg/L unionized	< 0.13 mg/L unionized	< 0.13 mg/L unionized
Aeration	Gentle, if D.O. falls below 60% saturation	Gentle, if D.O. falls below 60% saturation	Gentle, if D.O. falls below 60% saturation	Gentle, if D.O. falls below 60% saturation

[a]Project specific requirements may include full spectrum lighting.

2.3 Biological Requirements

Feeding None
Life stage Larval Stage used within 2 hours of fertilization depending on test species.

3.0 TEST ORGANISM

The test organism should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions, programmatic requirements, ecological importance, and ease of handling in the laboratory. For the larval test, availability of gravid adults often guides test species selection. The organisms for this protocol are *D. excentricus, S. purpuratus, Mytilus* sp.,and *C. gigas*.

3.1 Test Organism Specifications

Species:	*Dendraster excentricus*	*Strongylocentrotus purpuratus*	*Mytilus sp.*	*Crassostrea gigas*
Age:	Larval Stage used within 2 hours of fertilization	Larval Stage used within 2 hours of fertilization	Larval Stage used within 2 hours of fertilization	Larval Stage used within 2 hours of fertilization
Source:	A.K. Siewers, Santa Cruz, CA; Carlsbad Aquafarms, Carlsbad, CA NewFields, Port Gamble, WA	Carlsbad Aquafarms, Carlsbad, CA NewFields, Port Gamble, WA	A.K. Siewers, Santa Cruz, CA; Carlsbad Aquafarms, Carlsbad, CA Taylor Shellfish, Shelton, WA	A.K. Siewers, Santa Cruz, CA Taylor Shellfish, Shelton, WA

3.2 Test Organism Care

Records of the stock shipments will be kept, including original source, feeding regime, and holding water characteristics.

4.0 TEST SUBSTANCE

The test substance will be labeled, properly stored, and tracked by internal chain-of-custody procedures throughout its tenure at NewFields LLC. The test substance will not be heated, filtered, distilled, frozen, or otherwise altered without prior written consent by the Client.

The test substance is stored at 2 - 6°C in a secure and distinct storage area.

5.0 EQUIPMENT

5.1 Instrumentation/Equipment

Microprocessor-controlled recorder, and a digital thermometer
Light meter
DO meter and probe
Salinity meter and probe
pH meter and probe
Ammonia probe meter and ancillary supplies

Method of measuring total sulfides
1 L test chambers
Clean filtered seawater
Pipets
Miscellaneous labware (wash bottles, tally counters, culture bowls, etc.)
Centrifuge for collecting pore water
20 mL Scintillation vials
Syringe (to inject KCl into echinoderms) with 18-22 gauge needle
Pasteur pipets and bulbs
Ice bath or refrigerator
Compound microscope
Neubauer hemocytometer
Sedgwick-Rafter (or equivalent) counting cell (1 mL)
Small siphon hose (2ft. long, 3/16 - 1/4 in diameter)
Laboratory timer
Controlled temperature water bath or room
100-mL graduated cylinder
Perforated plunger

5.2 Reagents

0.5 M KCl
5% Buffered formalin
Copper sulfate
10% hydrochloric acid
Acetone
5% buffered formalin

5.3 Apparatus

5.3.1 Test Area

The test area consists of a room with constant temperature and appropriate illumination. Alternatively, a water table held at test temperature will be used to hold test chambers at test temperature. The facility will be well ventilated and free of fumes.

5.3.2 Lighting

Overhead lighting will be at 50-100 foot-candles (550-1050 Lux). In some cases, full spectrum lighting may be required, based on guidance provided in the Sediment Sampling and Analysis Plan Appendix, Subappendix D (Ecology 2008).

5.3.3 Test Chambers

1-L glass chambers
20 mL glass scintillation vials

6.0 PROCEDURE

Prior to use, all glassware and plasticware will be thoroughly cleaned.

6.1 Preparation

6.1.1 Glassware Preparation

Labware is described as any plastic or glass material used in the laboratory that will come into contact with any of the test substances or organisms in this evaluation. Labware must be cleaned prior to use. Labware will first be soaked in deionized water then scrubbed with a brush on all surfaces using non-phosphate detergent in deionized water. Alconox® is a widely used established brand of detergent used in laboratory applications. The clean materials will then be rinsed three times with running deionized water. Labware will then be allowed to soak in a 10% hydrochloric acid bath and afterwards rinsed three times with deionized water. Glass labware with also receive a solvent rinse with reagent grade acetone, and finally rinsed three times with deionized water. Some plastic labware is not resistant to solvents and may be damaged by acetone. Plastic labware such as Teflon can receive a solvent rinse, but all other plastics should be investigated prior to solvent rinsing.

6.1.2 Dilution Water Preparation

Filtered seawater collected from North Hood Canal will be diluted to 28 ± 1 ppt salinity using deionized water. Seawater will be held for a maximum of 2 days prior to use.

6.1.3 Test Organism Acclimation

Stock cultures will be acclimated in the same dilution water and at the same temperature as in the test procedures. Short-term culture logs will be maintained throughout the holding period.

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6.2 Primary Task

6.2.1 Test Sediment Addition

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

Eighteen grams of reference or test sediment is added to each of six 1-L glass test chambers pre-labelled with a randomly-assigned position number. 900 mL of filtered seawater (28 ppt salinity) is then added to each test chamber. A seawater-only control treatment is also prepared by placing 900-mL of test seawater into six clean, glass 1-L jars.

Once sediment and water have been placed into each of the test chambers, the sediment-water preparations are suspended by vigorously shaking each jar for 10 seconds. Test chambers are then placed into their randomly assigned position. The sediment-water mixtures are allowed to settle for four hours prior to embryo inoculation.

6.2.2 Reference Toxicant Test

Concurrent with this toxicity test, five concentrations of a reference substance (copper sulfate) are used to assess the health of the test organisms. Three replicates per reference concentration will be used. Five concentrations will be selected to bracket the 48-hour LC_{50}. The LC_{50} values will be compared with historical data from definitive bioassays. The results of this growth and survival test, conducted during this study, will be reported and used in combination with control growth and survival to characterize the health of the test organisms.

6.2.3 Test Organism Spawning and Addition

To collect gametes for testing with bivalve species, the adult organisms are placed in clean seawater and acclimated to test salinity (16°C for *Mytilus* and 20°C for *Crassostrea*) for approximately 20 minutes. The water bath temperature is then increased 5°C over a period of 15 minutes. Bivalves are maintained at this elevated temperature and monitored for spawning individuals. Spawning animals are removed from the water bath and placed in individual containers with seawater. Gametes from at least two males and two females are used to initiate the test. Once sufficient eggs and sperm had been collected, the eggs are screened though 500-µm mesh to remove any detritus or feces and a homogenized sperm solution added to the egg solutions. Egg-sperm solutions are periodically homogenized with a perforated plunger during the fertilization process. Approximately one hour after fertilization, embryo solutions

are checked for fertilization and cell division. Only those embryo stocks with >90% cell division are used to initiate the tests. Density of the embryo stock solution is determined by counting the number of embryos in a subsample of stock solution. For bivalve species, approximately 20,000 - 40,000 embryos will be added to test chambers within 2 hours of fertilization.

To collect gametes required for testing echinoderm species, spawning is induced in the adult organisms by the injection of 0.5 to 1.0 mL of 0.5 M KCl into the coelemic cavity through the perisotomal membrane. The injection is performed while the adult animal is out of water. Females will release orange (*S. purpuratus*) or purple (*D. excentricus*) eggs and males of both species will release cream-colored sperm. Once release has been initiated, each adult is inverted over a 50 to 100 mL beaker with filtered seawater at 15 degrees Celsius and gametes are allowed to accumulate for approximately 15 minutes. Once sufficient eggs and sperm had been collected, the eggs are transferred to a larger beaker with cold filtered seawater and a homogenized sperm solution (taken from several males) is added to the egg solutions. Egg-sperm solutions are periodically homogenized with a perforated plunger during the fertilization process. Approximately one half-hour after fertilization, embryo solutions are checked for fertilization. Only those embryo stocks with >90% fertilization are used to initiate the tests. Density of the embryo stock solution is determined by counting the number of embryos in a subsample of stock solution. Approximately 25,000 embryos will be added to test chambers within 2 hours of fertilization.

The test is initiated by randomly allocating an aliquot of the embryo stock solution into each test chamber four hours after sediments are shaken and within two hours of egg fertilization. Embryos are held in suspension during initiation using a perforated plunger. The test chambers are covered and incubated for 48 hours or longer under the conditions specified in Section 2.2.

For embryos/larvae test, a perforated plunger is used to mix the embryos/larvae at the test initiation. Approximately 25,000 echinoderm or 20,000-30,000 bivalve embryos will be added to test chambers within 2 hours of fertilization.

In order to determine the initial embryo concentration, 10-mL subsamples are collected from at least five randomly selected water quality surrogate chambers and preserved using 1 mL of 5% buffered formalin.

6.2.4 Test Initiation

The test is initiated when the first organism enters a test chamber.

6.2.5 Test Substance Renewal

No test substance renewals are required by this protocol.

6.2.6 Test Measurements

Water Quality. All probes will be cleaned thoroughly before initial use. Data collection will be performed on each samples respective surrogate chamber and recorded. The probes will be rinsed with de-ionized water between each sample. DO, temperature, salinity, and pH will be measured from the overlying water in the surrogate chamber daily. Ammonia and total sulfide should be measured in the overlaying water at least at the beginning of the test.

Biological. Larvae will be scored for normal development according to ASTM guidelines.

6.2.7 Test Termination

For echinoderm species, the test is terminated at 48 hours or when greater than 90% of the embryos in the duplicate seawater control have reached normal development (whichever is later and within 96 hours). For bivalve species, the test is terminated at 48 hours or when greater than 95% of the embryos in the seawater control have reached normal development (whichever is later and within 60 hours).

The water and the larvae overlying the settled sediment in each container are carefully mixed in order to suspend larvae and prevent disturbance of the sediment. The overlying water and larvae are then decanted into a clean, 1-L beaker. The contents of each chamber are then mixed using a perforated plunger, and a 10-mL subsample is removed and placed into a 20-mL scintillation vial. The contents of each vial are preserved with 1 mL of 5% buffered formalin, and the caps are securely replaced on the vials.

Preserved samples are examined on inverted microscope. Normal and abnormal larvae are enumerated to determine the percent normal survival, percent survival and percent normal. Normal larvae include all D-shaped prodissoconch I stage or pluteus larvae. Abnormal larvae include abnormally shaped prodissoconch I/pluteus larvae and all early stage larvae. Endpoints are calculated following formulas described in **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995)**, as well as updates provided during the Sediment Management Annual Review Meetings (SMARM).

6.2.8 Screen-Tube Test Modification

For some testing programs, the larval test may be conducted following a modified PSEP protocol, using a screen tube to separate physical effects of the sediment matrix from the potential effects of sediment-associated contaminants of concern. Modifications to the standard PSEP protocol are described below.

The screen tube is a 2" diameter Lexan® tube which is fitted at one end with a 25µm nytex screen. Prior to use, the screen tubes are washed according to procedures described in Section 6.1.1. However, the screen tubes are not rinsed in solvent, but are soaked overnight in clean seawater prior to use.

The test sediment and water are prepared in a manner similar to that described in Section 6.2.1. Control and reference treatments are tested concurrent with test treatments during the screen-tube tests. Both the control and reference treatments are tested with screen tubes.

Immediately prior to the introduction of larvae to the test chambers, the screen tubes are immersed in each test chamber. Screens should be gently placed in direct contact with the sediment surface. The top of the screen tube should be well above the surface of the water in the test chamber.

The test is initiated in a manner similar to that described in Section 6.2.3. However, for the screen-tube test, larvae are placed directly into the screen tube and are not inoculated in the surrounding test water. The stocking density in the screen tubes should be similar to that of the standard test method based on a screen tube volume of 270mL.

In order to determine the initial embryo concentration for the screen-tube test, five 10-mL subsamples should be collected from at least five randomly selected chambers using 1-mL of 5% buffered formalin. Water quality surrogate chambers should be used for determining stocking density, to prevent altering the total volumes in the test chambers. Prior to collecting the stocking density subsample, the contents of the screen tube should be homogenized using a perforated plunger.

Daily water quality should be monitored within the screen tube. At test termination, the contents each screen tube are homogenized using a perforated plunger and a 10-mL subsample collected, placed in a 20-mL scintillation vial, and preserved with 5% buffered formaldehyde.

The preserved subsamples are enumerated following the methods outlined in Section 6.2.7.

6.3 Post-task

Calculate the percent mortality for each replicate:

Mortality = 100 x (1-(No. of surviving test larvae / Mean No. of control larvae))

Calculate the percent abnormality for each replicate:

Abnormality = 100 x (1-(No. of abnormal larvae / No. of normal and abnormal survivors))

Calculate the combined larval mortality/abnormality:

Combined larval mortality/abnormality = 100 x (1-(No. of surviving normal larvae /mean No. of normal embryos in the control))

Results Needed:

- Individual replicate, mean, and standard deviation values for percent mortality, percent abnormality, and combined larval mortality/abnormality
- Plot of dose-response curve at test termination (48 or 72 hours)
- LC_{50} value for mortality, EC_{50} value for abnormality
- 95% confidence limits of LC_{50} value and EC_{50} value
- Tables showing biological, chemical, and physical data

6.3.1 Method

For the larval test, the normalized combined mortality and abnormality endpoint was used to evaluate the test sediment. This was based on the number of normal larvae in the treatment and reference divided by the number of normal larvae in the control, as defined in Ecology (2005).

For SMS suitability determinations, comparisons are made according to SAPA and Fox et al. (1998). Data reported as percent mortality or survival is transformed using an arcsine square root transformation prior to statistical analysis. All data are tested for normality using the Wilk-Shapiro test and equality of variance using Levene's test. Determinations of statistical significance are based on one-tailed Student's t-tests with an alpha level of 0.10. For samples failing to meet assumptions of normality, a Mann-Whitney test is conducted to determine significance. For those samples failing to meet the assumptions of normality and equality of variance, a t-test on rankits is used.

6.4 Reporting

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

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

The master copy of the final report will be signed and dated by the Study Director.

7.0 HEALTH AND SAFETY CONSIDERATIONS

Laboratory personnel will practice good personal sanitation and health practices, consistent with the objectives of this protocol.

Any health-related problem or other condition of laboratory personnel that may be considered to affect the study adversely will be reported to the Client.

Any injury to laboratory personnel obtained during this study, which may be considered related to the test material, will be reported to the Client.

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Every reasonable precaution will be taken to minimize exposure of personnel, the laboratory, and the environment to the test substance.

8.0 PERSONNEL

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

9.0 QUALITY ASSURANCE REQUIREMENTS (ACCEPTANCE CRITERIA)

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

Test acceptability criteria are:

- ≥ 90% survival of embryos introduced into control test chambers.
- ≥ 70% of embryos demonstrate normal development in the control.
- "The EC_{50} for the positive control test (copper sulfate) should be within the mean EC_{50} $\pm$ 2 standard deviations of the control chart" mean for the mussel embryo development test (Windward 2005, pg. 71).
- In the case of the failure of the negative control, toxicitiy test samples will be retested within the (8-week) holding time.

10.0 REFERENCE DOCUMENTS

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Norberg-King, T.J. 1988. An interpolation estimate for chronic toxicity: The ICp approach. Technical Report 05-88, National Effluent Toxicity Assessment Center, Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.

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USACE/USEPA (U.S. Army Corps of Engineers/U.S. Environmental Protection Agency). 1991. Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual." Office of Water, Washington, DC. EPA/503/8-911001. February, 1991.

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