US ARMY CORPS
OF ENGINEERS
New England District

Contract No. DACW33-01-D-0004
Delivery Order No. 02
August 2002

Final Report

AN EVALUATION OF THE TOXICITY OF SEDIMENTS TO TWO SPECIES OF MARINE AMPHIPODS

RHODE ISLAND REGION LONG-TERM DREDGED MATERIAL DISPOSAL SITE EVALUATION PROJECT

FINAL

An Evaluation of the Toxicity of Sediments to Two Species of Marine Amphipods

Rhode Island Region
Long-Term Dredged Material Disposal Site Evaluation Project

Contract Number DACW33-01-D-004
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to

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August 7, 2002

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INTRODUCTION

The Brenton Reef Disposal Site has been the only site in Rhode Island Sound extensively used for the disposal of dredged materials. The last disposal there occurred in the 1970s (Federal Register, 2001). Other potential disposal sites in Rhode Island Sound were identified during the 1980s, but were never used (Federal Register, 2001). Several studies within the last 20 years have indicated the need for a regional dredged material disposal site. The U.S. Environmental Protection Agency (US EPA Region 1) and the U.S. Army Corps of Engineers (the Corps) are considering the designation of a long-term disposal site in Rhode Island Sound and adjacent waters under section 102(c) of the Marine Protection, Research and Sanctuaries Act in a forthcoming EIS. As a part of studies being conducted for the EIS, EPA Region 1 and the Corps are evaluating the long-term consequences of dredged material disposal at Site 16 (also known as Breton Reef in other studies). The toxicity testing presented in this report is one part of that study.

Site 16 is located about 6 nm south of Newport, Rhode Island. Water depths at the site are shallowest (~25 m) in the vicinity of the disposal mound and deepest (~32 m) in areas that are probably the natural sea floor (SAIC 1990). Historical sedimentology, based on the results of a REMOTS® survey conducted in 1987 (SAIC 1990), showed that sediments near the apex of the mound exhibited considerable layering, with very fine to fine sand often layered over mud. Thickness of the dredged material at the mound varied from about 3 cm to 12 cm. Sediments graded to muds off the mound. In a relatively small area along the southeast border of the site, the sediments were fairly coarse (phi < -1), but were layered over mud. Disposal activity off the mound may have contributed to the development of this feature, or the sediments there may be indicative of natural conditions (SAIC 1990).

METHODS

Field Sampling Program

Five samples were collected from each of three Site 16 stations (Figure 1, Table 1). One sample was collected from Site 16 Reference station BRR1 (Figure 1, Table 1). Samples could not be collected successfully from Site 16 Reference stations BRR2 and BRR3 (Short and Albro 2002). Sampling at Site 16 to collect sediments for toxicity testing began September 13, 2001 (Table 1) with the collection of three samples from station BR1. Bad weather, rough seas, and sampling difficulties delayed the collection of additional toxicity samples until September 27–28, 2001, and then again until October 12, 2001.

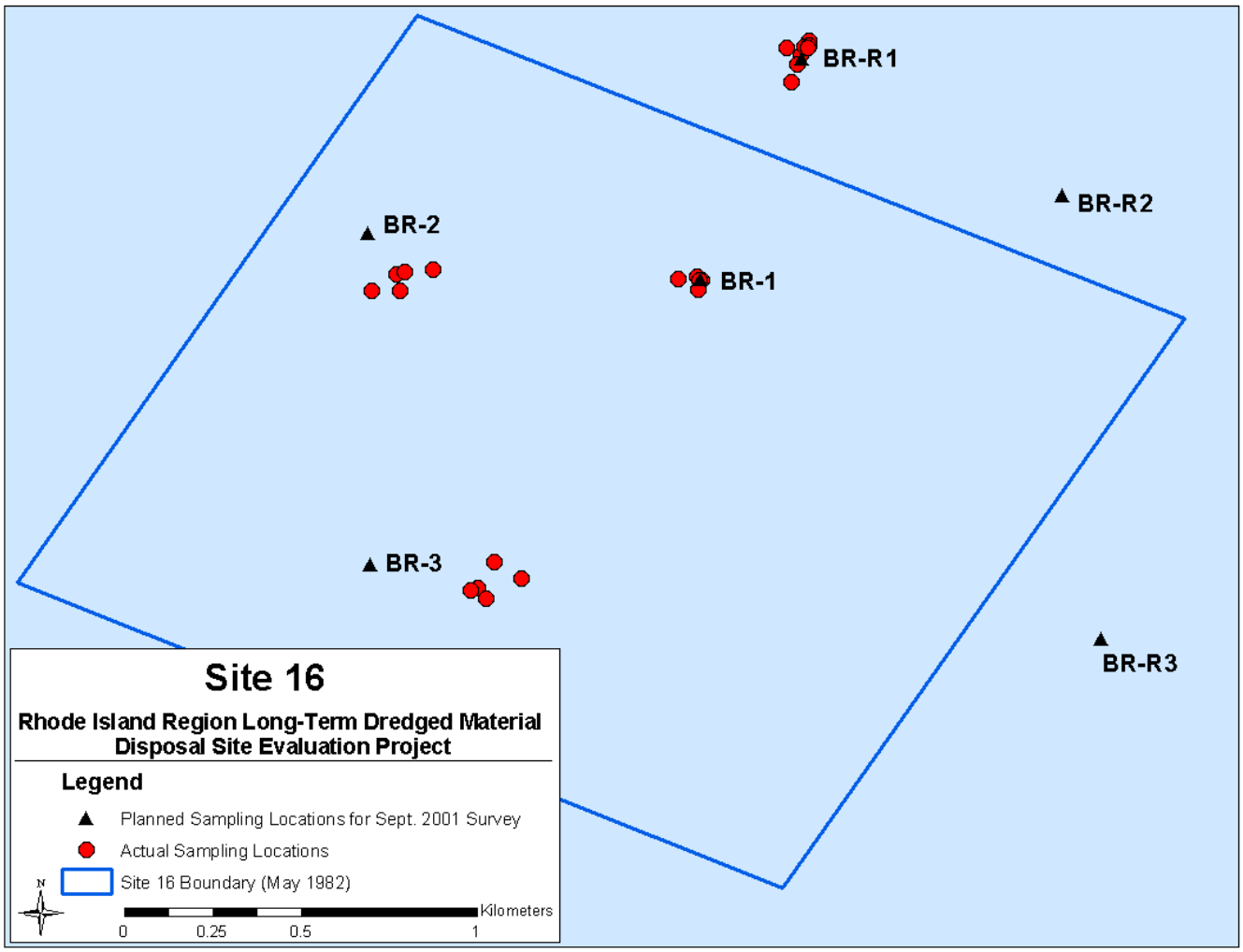

Figure 1. Locations of Stations within Site 16 Sampled for Use in the Toxicity Testing (from Short and Albro 2002).

Table 1. Site 16 Station Locations for Samples Used in the Toxicity Testing (from Short and Albro 2002).

Sample ID	Station Name	Latitude (°N)	Longitude (°W)	Sample Date/Time	Depth (m)
RIS1S269TX1	BR Reference	41.40127	71.29315	10/12/01 17:21	31.1
RIS1S1A1TX1	BR1	41.39542	71.29598	9/13/01 15:42	31.1
RIS1S1A6TX1	BR1	41.39535	71.29585	9/13/01 16:41	30.5
RIS1S1A9TX1	BR1	41.39535	71.29591	9/13/01 17:23	42.6
RIS1S23DTX1	BR1	41.39537	71.29646	10/12/01 9:05	30.5
RIS1S241TX1	BR1	41.39510	71.29594	10/12/01 9:58	30.7
RIS1S1B7TX1	BR2	41.39548	71.30363	9/27/01 10:05	29.5
RIS1S1EDTX	BR2	41.39562	71.30272	9/28/01 9:38	29.5
RIS1S1F0TX1	BR2	41.39507	71.30355	9/28/01 10:16	29.5
RIS1S1F7TX1	BR2	41.39508	71.30428	9/28/01 11:13	28.5
RIS1S1FBTX1	BR2	41.39555	71.30344	9/28/01 11:49	28.3
RIS1S1FETX1	BR3	41.38815	71.30115	9/28/01 12:29	29.6
RIS1S206TX1	BR3	41.38752	71.30157	9/28/01 13:29	29.5
RIS1S20ATX1	BR3	41.38745	71.30173	9/28/01 14:05	29.5
RIS1S20FTX1	BR3	41.38723	71.30135	9/28/01 14:50	29.6
RIS1S212TX1	BR3	41.38773	71.30047	9/28/01 15:22	30.0

Samples were collected using a 0.25-m^2-Mark III Sandia box corer device. The box corer provided enough surface area, in one or two drops, to provide 1 to 2 gallons of sediment, removed from the top two centimeters of the sample, for analysis of the targeted physical and chemical analytes, samples for acid volatile sulfides (AVS) and simultaneously extracted metals (SEM), benthic infauna, and also for toxicity testing. It also ensured that samples for use in the sediment quality triad analyses (chemical constituents, benthic infauna, and toxicity) were synoptically collected. Additional information about the sampling procedures and survey are contained in the survey report (Short and Albro 2002).

Amphipod Toxicity Tests

Bioassay testing of Site 16, Rhode Island Sound (hereafter referred to as Site 16) sediments followed guidance provided in *Evaluation of Dredged Material Proposed for Ocean Disposal—Testing Manual* (EPA/USACE 1991), also referred to as the "Green Book," and the *Guidance for Performing Tests on Dredged Material to be Disposed of in Open Waters* (EPA/USACE 1989), which provides regional guidance for EPA Region 1 and the USACE, New England District.

Because of the presence of fine- and coarse-grained sediments within Site 16, two species of test organisms were used to evaluate solid phase toxicity of Site 16 samples:

- *Ampelisca abdita*, a tube-building amphipod typically used for testing fine-grained sediments
- *Eohaustorius estuarius*, a burrowing amphipod typically used for testing coarse-grained sediments

Test organisms and some control sediment samples were obtained from commercial suppliers. *Ampelisca abdita* and its control sediment were supplied by Eastern Aquatic Biosupply of Middletown, Rhode Island. *Eohaustorius estuarius* and its control sediment were supplied by Northwest Aquatics of Newport, Oregon.

All test organisms were delivered to Marine Sciences Laboratory (MSL) in sediment under appropriate conditions to ensure viability. Upon arrival at MSL, the test organisms were acclimated slowly to test conditions. During holding, *Ampelisca abdita* and *Eohaustorius estuarius* were fed an algal concentrate. Organisms exhibiting abnormal behavior or appearance during acclimation or initiation were not used in toxicity tests. Receipt, acclimation, and animal care records are part of the data files for this project.

Sediment Sample Preparation

Eighteen sediment samples were prepared for the Site 16 solid-phase acute toxicity tests: fifteen harbor test composites, one reference sediment, and two control sediments. Upon receipt at Battelle MSL, temperature of the sediments was measured and a sediment subsample was collected for porewater ammonia concentration analysis.

A. abdita and *E. estuarius* control sediments were sieved through 0.5-mm-mesh sieves to remove any live organisms that might interfere with the test. All sediment samples were stored in MSL's walk-in cold room at 4 ± 2 ºC until used in toxicity or bioaccumulation tests.

At MSL, small sediment samples were collected for porewater ammonia analyses. During this process, several samples were observed to contain living ampeliscid amphipods. Because these amphipods could confound the results of the testing with *A. abdita*, the New England District Corps of Engineers (NED) project manager and the EPA representative were notified. It was decided that the test sediments to be used in the *A. abdita* testing would be press-sieved (dry) through a 1.0-mm-mesh sieve. After the test sediments were layered into the test jars and placed on the water table, the NED and EPA expressed concern that there still may be live *A. abdita* in the sediments that could confound the test results. A decision was made to delay test initiation one day to allow the sediments to be examined for the presence of *A. abdita*. Small subsamples from each sediment that had material remaining in cold storage were obtained examined for the presence of live amphipods. Any live specimens of *Ampelisca* remaining in the subsamples were identified to ensure that no *A. abdita* were present. Samples that had been placed on the wet tables for testing could not be examined without compromising the testing conditions.

Amphipod Acute Toxicity Test Preparation

Benthic acute toxicity testing using the marine amphipods *Ampelisca abdita*, and *Eohaustorius estuarius* was performed at Battelle MSL. Grain-size data from samples collected in September 2001 were used to determine which species would be used to test the sediments (Table 2). *A. abdita* were exposed to 12 test site composites with relatively fine grain sizes plus the Site 16 Reference Site sediment. *E. estuarius* were exposed to four test site composites with relatively course grain sizes plus the Site 16 Reference Site sediment. One of the sediments from Station BR2 that was to be tested with *E. estuarius* was also tested with *A. abdita*. As a test of its ability to survive an acute toxicity test in relatively coarse sediments, MSL also exposed *A. abdita* to the three high-gravel sediments that were being tested with *E. estuarius*. Tests with both species were 10-day static (i.e., without renewal of water in test containers) or static-renewal (with periodic, scheduled renewal of water in test containers) exposures with mortality as the endpoint.

Table 2. Grain-size Distribution and Species Tested for Sediments from Site 16.

Sample ID	Station ID	Species Tested	Percent (%) Sediment Grain-Size Distribution						
					Sand				
			Cobble	Gravel	Coarse	Medium	Fine	Silt	Clay
RIS1S269	BR Reference	*A. abdita*, *E. estuarius*	0.00	0.00	0.68	2.29	31.57	45.46	20.00
RIS1S1A1	BR1	*A. abdita*	0.00	0.36	0.94	9.16	52.60	24.70	12.25
RIS1S1A6	BR1	*A. abdita*	0.00	0.30	0.28	3.89	56.04	27.00	12.50
RIS1S1A9	BR1	*A. abdita*	0.00	0.00	0.24	3.31	49.07	31.87	15.50
RIS1S23D	BR1	*A. abdita*	0.00	0.29	0.77	4.71	55.00	25.24	14.00
RIS1S241	BR1	*A. abdita*	0.00	1.29	0.64	4.60	61.28	21.19	11.00
RIS1S1B7	BR2	*A. abdita*	0.00	0.34	0.49	8.33	68.21	14.63	8.00
RIS1S1ED	BR2	*A. abdita*, *E. estuarius*	0.00	4.73	8.02	53.33	28.94	1.48	3.50
RIS1S1F0	BR2	*A. abdita*	0.00	0.00	0.13	6.80	87.40	2.35	3.32
RIS1S1F7	BR2	*A. abdita*	0.00	4.74	9.66	65.65	15.90	0.55	3.50
RIS1S1FB	BR2	*A. abdita*	0.00	0.00	0.10	8.62	86.88	1.29	3.11
RIS1S1FE	BR3	*A. abdita*	0.00	1.48	0.55	7.68	83.27	3.03	4.00
RIS1S206	BR3	*A. abdita*	0.00	0.32	0.80	7.29	76.50	9.08	6.00
RIS1S20A	BR3 [1]	*E. estuarius*	0.00	15.37	15.62	53.68	11.15	1.15	3.03
RIS1S20F	BR3 [1]	*E. estuarius*	0.00	23.65	17.71	29.06	21.39	3.70	4.50
RIS1S212	BR3 [1]	*E. estuarius*	0.00	25.59	12.43	33.17	22.21	2.77	3.83

[1] As an addition to the overall study, these sediments were also tested using *A. abdita.*

Ammonia purging and monitoring followed the guidance in *Methods for Assessing the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Amphipods* (EPA 1994a), which calls for aerating the sample and replacing up to two volumes of overlying water per day if ammonia values exceed species thresholds. The porewater ammonia threshold for *A. abdita* is 20 mg/L total ammonia. During the *A. abdita* bioassay, ammonia was measured in the porewater and the overlying water of surrogate test chambers on Test Days 0, 3, and 10.

The tests were initiated as soon as possible after ammonia levels in all treatments were determined to be below thresholds. Test chambers for both tests were 1-L glass (Mason) jars modified to permit seawater flow-through during ammonia purging operations. Test chambers were fitted with air and water flow delivering lids, and screened overflow ports. The *Ampelisca* test was initiated on November 7, 2001 by introducing 20 organisms into each test chamber. To maintain low ammonia levels during the test, the overlying water in each test chamber was exchanged once per day. The *Eohaustorius* test was initiated on November 5, 2001 by introducing 20 organisms into each test chamber.

For both tests, five replicates of each test sediment were initiated. Water quality parameters (temperature, pH, dissolved oxygen, and salinity) were measured in all replicates of each test prior to test initiation, in one replicate per day during the test, and again in all replicates prior to test termination. Observations of conditions in each chamber were made daily. The amphipods were not fed during testing. All bioassays included a negative (laboratory) control sediment to validate the test, and a concurrent 96-h, water-only, reference toxicant test to assess the sensitivity of each test population. Target test conditions for the benthic toxicity tests are provided in Table 3.

Water-only reference toxicant tests (96 h) were initiated concurrently with the solid phase tests. Procedures followed those for the sediment tests, but test chambers were run without water renewals for the entire test duration. Cadmium was the toxicant for the *A. abdita* and *E. estuarius* tests. Test concentrations are listed in Table 3.

Toxicity Data Analysis and Interpretation

Each test was based on a random design to allow unbiased comparison between treatments. Organisms were randomly allocated to treatments, and treatment replicates were randomly positioned on water tables. Random positions for test chambers were assigned using the discrete random-number generator in Microsoft Excel spreadsheet software.

To be defined as acutely toxic, the response (survival) in the test sediments must differ from that in the reference sediments by more than 20% (for *A. abdita*) or 10% (for *Eohaustorius*) and must be significantly lower (statistically) than the appropriate reference treatment response.

Table 3. Target Conditions for the Amphipod Solid-Phase Acute Toxicity Tests.

Parameter	*Eohaustorius estuarius*	*Ampelisca abdita*
Duration	10 days	10 days
Treatments	4 Brenton Reef test treatments 1 Brenton Reef Reference *E. estuarius* control	12 Site 16 test treatments (3 high-gravel Brenton Reef treatments) 1 Site 16 Reference *A. abdita* control
Replicates	5, plus surrogates for ammonia monitoring	5, plus surrogates for ammonia monitoring
Test population	20 individuals per replicate, total n = 100 (per treatment)	20 individuals per replicate, total n = 100 (per treatment)
Flow Regime	Static (1–2 exchanges per day if ammonia exceeds threshold)	Static renewal, 1 exchange per day (2 exchanges per day during purging)
Temperature	15°C ± 2°C	20 ± 2 °C
Lighting:	Continuous	Continuous
Dissolved Oxygen	> 3.4 mg/L (=40% saturation at 15 ºC, 30‰)	> 4.6 mg/ (= 60% saturation at 20 ºC, 30‰)
pH	7.8 ± 0.5	7.8 ± 0.5
Salinity	30 ± 2 ‰	30 ± 2 ‰
Ammonia	<60 mg/L total ammonia in porewater at test initiation	<20 mg/L total ammonia in porewater at test initiation
Feeding	None during test	None during test
Reference Toxicant	Cadmium at 0, 3.5, 7, 14, 28 mg/L	Cadmium at 0, 0.25, 0.5, 1, and 2 mg/L
10-day Test Validity Criteria	≥ 90% survival in control sediments	≥ 90% survival in control sediments

RESULTS

Acid Volatile Sulfides, Simultaneously Extracted Metals

Acid Volatile Sulfides (AVS)/Simultaneously Extracted Metals (SEM) analysis is commonly used in estimating toxicity. Sediments samples from Rhode Island Sound were analyzed for AVS/SEM as described in Battelle, 2002. AVS concentrations determined for most of the Site 16 sediments used in the toxicity testing were below the method detection limits or otherwise very low (Table 4). Therefore, AVS provides no information to explain the toxicity, or lack thereof, of the Site 16 sediments. The results of the SEM analysis are discussed in Battelle, 2002.

Table 4. Acid Volatile Sulfide and Simultaneously Extracted Metal Concentrations of the Site 16 Sediments Used in the Toxicity Testing Conducted in November 2001.

			SEM					
Station	Sample_ID	AVS µmole/g	Cadmium µmole/g	Copper µmole/g	Lead µmole/g	Nickel µmole/g	Silver µmole/g	Zinc µmole/g
BR1	RIS1S1A1	*0.68* [1]	NC [2]	0.109	0.0437	0.0196	0.000596	0.255
BR1	RIS1S1A6	*0.56*	NC	0.119	0.0486	0.0196	0.000967	0.206
BR1	RIS1S1A9	*0.72*	NC	0.123	0.0588	0.0328	0.000916	0.267
BR1	RIS1S23D	*1.47*	NC	0.109	0.0563	NC	0.000670	0.233
BR1	RIS1S241	*1.06*	NC	0.100	0.0496	NC	0.000669	0.229
BR2	RIS1S1B7	*0.522*	NC	0.0622	0.0364	0.0132	0.000388	0.168
BR2	RIS1S1ED	*0.164*	NC	0.0130	0.0109	0.00762	0.000051	0.057
BR2	RIS1S1F0	*0.053*	NC	0.0204	0.0158	NC	0.000232	0.069
BR2	RIS1S1F7	*0.045*	NC	0.0147	0.0121	0.00809	0.000108	0.049
BR2	RIS1S1FB	*0.055*	NC	0.0147	0.0136	NC	0.000161	0.068
BR3	RIS1S1FE	*0.053*	NC	0.0330	0.0203	NC	0.000256	0.075
BR3	RIS1S206	*0.088*	NC	0.0589	0.0272	0.00782	0.000511	0.100
BR3	RIS1S20A	0.160	NC	0.0213	0.0119	NC	0.000156	0.065
BR3	RIS1S20F	0.203	NC	0.0622	0.0239	0.0105	0.000325	0.112
BR3	RIS1S212	*0.055*	NC	0.0316	0.0154	0.0128	0.000291	0.063
BRR1	RIS1S269	2.08	NC	0.0711	0.0569	0.0145	0.000428	0.264
BRR1	RIS1S269	2.14	NC	0.0665	0.0493	0.0147	0.000872	0.237

[1] Italicized values were below the method detection limits.
[2] NC, No calculation performed; µg/g concentration less than the method detection limit.

Amphipod Acute Toxicity Tests

Ammonia Measurements

Bulk-sediment-porewater ammonia measurements showed that ammonia thresholds were not exceeded for either amphipod test species, therefore, purging was not required to reduce porewater ammonia prior to testing. This information is presented in Tables 5 and 6, including the measured ammonia levels. For the *Ampelisca* test, sediments were layered on November 5, 2001, two days before the test was initiated. For the *Eohaustorius* test, sediments were layered on November 4, 2001, one day before the test was initiated. Note that the Day 10 porewater ammonia measured for sample RIS1S20FTX1 (Station BR3) was 67 mg/L. A dead, 2.5-cm long

clam (*Arctica islandica*) was in the surrogate test jar for this sediment and was responsible for the high ammonia concentration measured.

Table 5. Ammonia Monitoring Data for the 10-day Solid-Phase Acute Toxicity Test with *Ampelisca abdita*, Site 16, November 2001.

			Ammonia in Porewater (mg/L)			
Sample ID	**Station ID**	**Date Layered**	**Bulk Sediment 23 Oct**	**Day 1 6 Nov**	**Day 3 10 Nov**	**Day 10 17 Nov**
A. abdita Control	–	11/5/01	NM [1]	13	6.5	1.2
RIS1S269TX1	BR Reference	11/5/01	5.8	14	3.7	1.7
RIS1S1A1TX1	BR1	11/5/01	2.5	4.1	1.9	1.5
RIS1S1A6TX1	BR1	11/5/01	3.2	5.8	2.1	1.2
RIS1S1A9TX1	BR1	11/5/01	*0.7* [2]	2.1	NM	*0.5*
RIS1S23DTX1	BR1	11/5/01	4.5	14	3.7	2.0
RIS1S241TX1	BR1	11/5/01	5.4	12	4.0	1.5
RIS1S1B7TX1	BR2	11/5/01	13	16	7.7	2.3
RIS1S1EDTX1	BR2	11/5/01	10	NPE [3]	NM	*0.2*
RIS1S1F0TX1	BR2	11/5/01	1.9	11	1.6	*0.9*
RIS1S1F7TX1	BR2	11/5/01	2.2	3.0	NPE	*0.9*
RIS1S1FBTX1	BR2	11/5/01	3.4	8.1	NPE	*0.8*
RIS1S1FETX1	BR3	11/5/01	5.1	13	NPE	1.0
RIS1S206TX1	BR3	11/5/01	14	NPE	1.5	1.7

[1] NM, Not measured
[2] Italicized numbers indicate estimated value, below the instrument calibration range
[3] NPE, No porewater could be extracted from sample.

Table 6. Ammonia Monitoring Data for the 10-day Solid-Phase Acute Toxicity Test with *Eohaustorius estuarius*, Site 16, November 2001.

			Ammonia in Porewater (mg/L)			
Sample ID	**Station ID**	**Date Layered**	**Bulk Sediment 23-Oct**	**Day 0 23-Oct**	**Day 3 26-Oct**	**Day 10 2-Nov**
E. estuarius Control	–	11/4/01	NM [1]	3.9	3.8	1.0
RIS1S269TX1	BR Reference	11/4/01	5.8	8.0	6.2	2.5
RIS1S1EDTX1	BR2	11/4/01	10	11	6.4	1.3
RIS1S20ATX1	BR3	11/4/01	5.9	5.4	3.3	*0.4* [2]
RIS1S20FTX1	BR3	11/4/01	22	11	10	67 [3]
RIS1S212TX1	BR3	11/4/01	10	9.2	3.2	19

[1] NM, Not measured
[2] Italicized numbers indicate estimated value, below the instrument calibration range
[3] A dead, 2.5-cm long *Arctica islandica* clam was found in the porewater sediment sample.

Ampelisca abdita 10-day Solid-Phase Test

Prior to initiating the toxicity test, sediment subsamples were examined for the presence of *A. abdita*. Although a few live amphipods, including a species of *Ampelisca*, were found in some sediments, none were identified as *A. abdita*. The species of *Ampelisca* found in the sediments was identified as *A. agassizi*, which is easily distinguished from *A. abdita*. Several specimens of the species were fixed in 10% formalin and archived at MSL. One species of corophiid amphipod, which was not identified to species, also was found in the samples. During the test termination, all specimens of *Ampelisca* collected from each test container were examined microscopically to ensure that only *A. abdita* were tallied in the reporting of the test results. One jar (replicate 5) of the Site 16 Reference sediment was inadvertently not initiated.

Results of the solid-phase test with *A. abdita* are summarized in Table 7. The complete test results by replicate are presented in Appendix A. Mean survival in the *A. abdita* control sediments was 97%, which validates the test. *A. abdita* mean survival in the Brenton Reef Reference sediments was 96%.

Water quality parameters (Appendix A) were within the acceptable ranges during the test except for minor deviations in salinity (greater than the upper limit by < 1 ‰). These deviations had no apparent effect on test results.

The cadmium reference toxicant results (Appendix A) showed that the test population was as sensitive as typical *A. abdita* populations that have been tested at MSL. The LC_{50} (0.67 mg/L) was within the control range of 0.34–1.23 mg/L (mean = 0.79 mg/L) as determined for the previous 20 tests of this species conducted at MSL.

Among the 12 Site 16 fine-grained test sediments, *A. abdita* mean survival ranged from 92% to 100% (Table 7). The absolute difference in survival between any test sediment versus the reference sediment did not exceed 20 %. Therefore, no statistical analyses were conducted. No Site 16 sediments were acutely toxic to *Ampelisca abdita*.

Table 7. Mean and Standard Deviation Survival in the 10-day Solid-Phase *Ampelisca abdita* Acute Toxicity Test, Site 16, November 2001.

Sample ID	Station ID	Percent Survival		Absolute Difference from Brenton Reef Reference (%)
		Mean	Standard Deviation	
A. abdita Control	–	97	7	NA
RIS1S269TX1 [1]	BR Reference	96	9	NA
RIS1S1A1TX1	BR1	98	4	+2
RIS1S1A6TX1	BR1	97	3	+1
RIS1S1A9TX1	BR1	100	0	+4
RIS1S23DTX1	BR1	95	5	–1
RIS1S241TX1	BR1	96	4	0
RIS1S1B7TX1	BR2	99	2	+3
RIS1S1EDTX1	BR2	92	6	–4
RIS1S1F0TX1	BR2	99	4	+3
RIS1S1F7TX1	BR2	92	3	–4
RIS1S1FBTX1	BR2	95	4	–1
RIS1S1FETX1	BR3	96	4	0
RIS1S206TX1	BR3	99	2	+3

[1] Laboratory replicate 5 was not initiated; mean and standard deviation are based on $n = 4$.

Eohaustorius estuarius 10-day Solid-Phase Test

Results of the solid-phase test with *E. estuarius* are summarized in Table 8. Complete test results by replicate are presented in Appendix B. Mean survival in the *E. estuarius* control sediments was 95%, which validates the test. *E. estuarius* mean survival in the Site 16 reference sediments was 92%.

Water quality parameters (Appendix B) were within the acceptable ranges during the test except for minor deviations in salinity (greater than the upper limit by < 1 ‰).

The cadmium reference toxicant results (Appendix B) showed that the test population was about as sensitive as typical *E. estuarius* populations that have been tested at MSL. The LC_{50} (11.2 mg/L) was within the control range of 3.0–18.2 mg/L (mean = 10.6 mg/L) as determined for the previous 20 tests of this species conducted at MSL. However, survival in the control concentration (0 mg/L) was 78%.

E. estuarius mean survival in the four Site 16 test sediments ranged from 87% to 92% (Table 8). The absolute difference in survival between any test sediment versus the reference sediment did not exceed 10 %. Therefore, no statistical analyses were conducted. No Site 16 sediments were acutely toxic to *Eohaustorius estuarius*.

Table 8. Mean and Standard Deviation Survival in the 10-day Solid-Phase *Eohaustorius estuarius* Acute Toxicity Test, Site 16, November 2001.

Sample ID	Station ID	Percent Survival		Absolute Difference from Brenton Reef Reference (%)
		Mean	Standard Deviation	
E. estuarius Control	–	95	4	NA
RIS1S269TX1	BR Reference	92	10	NA
RIS1S1EDTX1	BR2	92	4	0
RIS1S20ATX1	BR3	92	6	0
RIS1S20FTX1	BR3	87	8	–5
RIS1S212TX1	BR3	92	7	0

Ampelisca abdita Tested in High-gravel Sediments

The three high-gravel sediments (see Table 2), all from station BR3, that were tested with *E. estuarius* were also tested with *A. abdita*. The five replicates for each of these three sediments were randomized as part of the experiment testing the fine-grained sediments with *A. abdita*. Because this experiment was conducted as part of the design for the primary *A. abdita* experiment and used animals from the same batch, the reference toxicant test results and the results for the Control and Reference sediments as reported previously are applicable here. Among the three high-gravel sediments that were also tested with *A. abdita* as an addition to the experiment, survival was very good, ranging from 91% to 98% (Appendix A; Table 9).

Table 9. Mean and Standard Deviation Survival in the 10-day Solid-Phase *Ampelisca abdita* Acute Toxicity Test of High-gravel Sediments, Site 16, November 2001.

Sample ID	Station ID	Percent Survival		Absolute Difference from Brenton Reef Reference (%)
		Mean	Standard Deviation	
Control	–	97	7	NA
Brenton Reef Reference	BR Reference	96	9	NA
RIS1S20ATX1	BR3	95	4	–1
RIS1S20FTX1	BR3	98	3	+2
RIS1S212TX1	BR3	91	10	–5

Although these three sediments had a high gravel component, they also had a fine sediment component, with silt+clay values ranging from 4.2% to 8.2%. The fine component was a very noticeable feature of the sediment surface after the sediments had been placed into the jars prior to testing (Figure 2). *A. abdita* tubes comparable to those seen in the control sediments (Figure 3) were noticeable in the three coarse sediments (Figure 4).

Figure 2. View of the sediment surface in one replicate container for the high-gravel sediment from Brenton Reef station BR3. An *A. abdita* tube is visible between the two rocks in the lower part of the photograph; two others are visible near the edge of the test jar just above the 9 o'clock position.

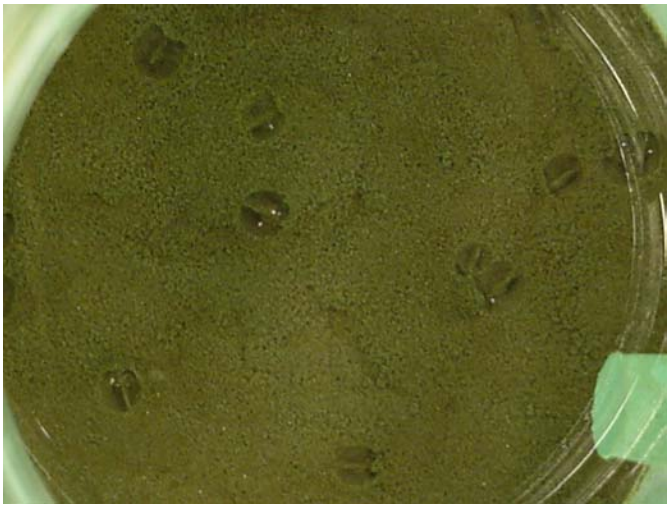

Figure 3. *A. abdita* tubes are easily visible as coffee bean-shaped patterns in a control sediment replicate during the Brenton Reef toxicity testing, November 2001.

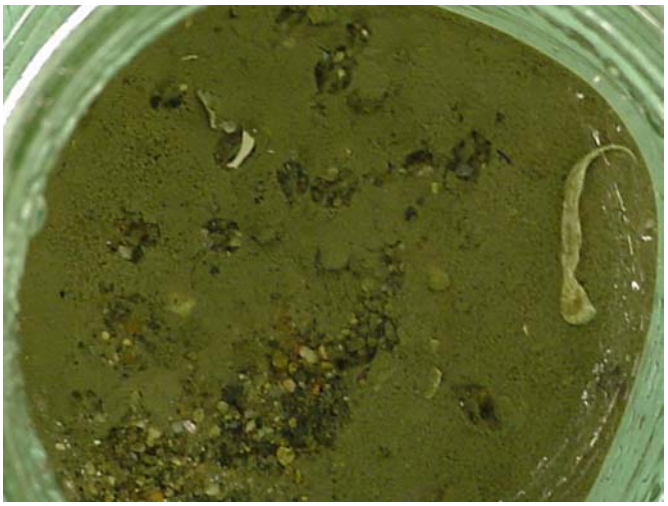

Figure 4. View of the surface of one replicate of a high-gravel sediment (Brenton Reef station BR3) showing several *A. abdita* tubes in the fine sediments among the coarse material.

CONCLUSIONS

The results of the toxicity testing program conducted on sediments collected from the historic Site 16 disposal site in 2001 showed that none of the sediments was acutely toxic to the marine amphipods *Eohaustorius estuarius* or *Ampelisca agassizi*. Additionally, supplemental tests showed that *Ampelisca agassizi* could survive in very coarse sediments (gravel + coarse sand content from 31.0% to 41.4%). However, these sediments also had a fine component (silt + clay ranging from 4.2% to 8.2%). The fine materials settled on the surface of the test containers allowing the amphipods to build tubes sufficient for their survival.

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APPENDIX A

10-day Solid Phase Test with *Ampelisca abdita*

Table A-1. Results of the 10-day solid phase test using *Ampelisca abdita*, Site 16, Rhode Island Sound sediments, November 2001.

Sample ID	Station ID	Rep	Number Alive	Number Dead or Missing	Proportion Surviving	Proportion Surviving		
						Mean	Standard Deviation	Coefficient of Variation
RIS1S1A1TX1	BR1	1	20	0	1.00			
RIS1S1A1TX1	BR1	2	20	0	1.00			
RIS1S1A1TX1	BR1	3	20	0	1.00			
RIS1S1A1TX1	BR1	4	18	2	0.90			
RIS1S1A1TX1	BR1	5	20	0	1.00	0.98	0.04	5%
RIS1S1A6TX1	BR1	1	20	0	1.00			
RIS1S1A6TX1	BR1	2	20	0	1.00			
RIS1S1A6TX1	BR1	3	19	1	0.95			
RIS1S1A6TX1	BR1	4	19	1	0.95			
RIS1S1A6TX1	BR1	5	19	1	0.95	0.97	0.03	3%
RIS1S1A9TX1	BR1	1	20	0	1.00			
RIS1S1A9TX1	BR1	2	20	0	1.00			
RIS1S1A9TX1	BR1	3	20	0	1.00			
RIS1S1A9TX1	BR1	4	20	0	1.00			
RIS1S1A9TX1	BR1	5	20	0	1.00	1.00	0.00	0%
RIS1S1B7TX1	BR2	1	20	0	1.00			
RIS1S1B7TX1	BR2	2	19	1	0.95			
RIS1S1B7TX1	BR2	3	20	0	1.00			
RIS1S1B7TX1	BR2	4	20	0	1.00			
RIS1S1B7TX1	BR2	5	20	0	1.00	0.99	0.02	2%
RIS1S1EDTX1	BR2	1	19	1	0.95			
RIS1S1EDTX1	BR2	2	17	3	0.85			
RIS1S1EDTX1	BR2	3	18	2	0.90			
RIS1S1EDTX1	BR2	4	18	2	0.90			
RIS1S1EDTX1	BR2	5	20	0	1.00	0.92	0.06	6%
RIS1S1F0TX1	BR2	1	20	0	1.00			
RIS1S1F0TX1	BR2	2	19	1	0.95			
RIS1S1F0TX1	BR2	3	20	0	1.00			
RIS1S1F0TX1	BR2	4	21 [1]	0	1.00			
RIS1S1F0TX1	BR2	5	19	1	0.95	0.99	0.04	4%
RIS1S1F7TX1	BR2	1	18	2	0.90			
RIS1S1F7TX1	BR2	2	19	1	0.95			
RIS1S1F7TX1	BR2	3	18	2	0.90			
RIS1S1F7TX1	BR2	4	18	2	0.90			
RIS1S1F7TX1	BR2	5	19	1	0.95	0.92	0.03	3%
RIS1S1FBTX1	BR2	1	19	1	0.95			
RIS1S1FBTX1	BR2	2	19	1	0.95			
RIS1S1FBTX1	BR2	3	19	1	0.95			
RIS1S1FBTX1	BR2	4	18	2	0.90			
RIS1S1FBTX1	BR2	5	20	0	1.00	0.95	0.04	4%

Table A-1. (continued)

Sample ID	Station ID	Rep	Number Alive	Number Dead or Missing	Proportion Surviving	Proportion Surviving		
						Mean	Standard Deviation	Coefficient of Variation
RIS1S1FETX1	BR3	1	19	1	0.95			
RIS1S1FETX1	BR3	2	20	0	1.00			
RIS1S1FETX1	BR3	3	18	2	0.90			
RIS1S1FETX1	BR3	4	20	0	1.00			
RIS1S1FETX1	BR3	5	19	1	0.95	0.96	0.04	4%
RIS1S206TX1	BR3	1	20	0	1.00			
RIS1S206TX1	BR3	2	20	0	1.00			
RIS1S206TX1	BR3	3	20	0	1.00			
RIS1S206TX1	BR3	4	20	0	1.00			
RIS1S206TX1	BR3	5	19	1	0.95	0.99	0.02	2%
RIS1S23DTX1	BR1	1	18	2	0.90			
RIS1S23DTX1	BR1	2	18	2	0.90			
RIS1S23DTX1	BR1	3	19	1	0.95			
RIS1S23DTX1	BR1	4	20	0	1.00			
RIS1S23DTX1	BR1	5	20	0	1.00	0.95	0.05	5%
RIS1S241TX1	BR1	1	20	0	1.00			
RIS1S241TX1	BR1	2	20	0	1.00			
RIS1S241TX1	BR1	3	19	1	0.95			
RIS1S241TX1	BR1	4	18	2	0.90			
RIS1S241TX1	BR1	5	19	1	0.95	0.96	0.04	4%
RIS1S269TX1 [2]	BRRF	1	20	0	1.00			
RIS1S269TX1	BRRF	2	19	1	0.95			
RIS1S269TX1	BRRF	3	17	3	0.85			
RIS1S269TX1	BRRF	4	21 [1]	0	1.00			
RIS1S269TX1	BRRF	5	– [3]	–	–	0.96	0.09	9%
Control	–	1	20	0	1.00			
Control	–	2	20	0	1.00			
Control	–	3	20	0	1.00			
Control	–	4	21 [1]	0	1.00			
Control	–	5	17	3	0.85	0.97	0.07	7%

[1] All identified as *A. abdita*; assumed that 21 amphipods were placed in jar at initiation.
[2] Reference sediment.
[3] This replicate was not initiated, therefore the mean and sd are based on $n = 4$.

Table A-2. Summary of Water Quality Measurements for the 10-day Solid Phase Test Using *Ampelisca abdita*, Site 16, Rhode Island Sound Sediments, November 2001.

Sample ID	Station ID	Temperature (° C)		Salinity [1] (‰)		Dissolved Oxygen (mg/L)		pH (units)	
		Min	Max	Min	Max	Min	Max	Min	Max
	target range:	18.0	22.0	28.0	32.0	4.6	NA [2]	7.3	8.3
RIS1S1A1TX1	BR1	19.5	20.4	31.6	32.4	6.7	7.5	7.5	7.9
RIS1S1A6TX1	BR1	19.4	20.3	31.6	32.2	6.6	7.5	7.5	7.9
RIS1S1A9TX1	BR1	19.4	20.3	31.6	32.3	6.5	7.5	7.5	7.9
RIS1S1B7TX1	BR2	19.4	20.3	31.6	32.4	6.5	7.5	7.5	7.9
RIS1S1EDTX1	BR2	19.5	20.2	31.6	32.3	6.6	7.5	7.5	7.9
RIS1S1F0TX1	BR2	19.4	20.3	31.6	32.4	6.3	7.5	7.5	7.9
RIS1S1F7TX1	BR2	19.4	20.3	31.6	32.3	6.1	7.5	7.4	8.0
RIS1S1FBTX1	BR2	19.4	20.4	31.7	32.4	6.6	7.5	7.6	8.0
RIS1S1FETX1	BR3	19.5	20.3	31.6	32.4	6.6	7.5	7.5	8.0
RIS1S206TX1	BR3	19.4	20.3	31.5	32.3	6.6	7.5	7.5	7.9
RIS1S23DTX1	BR1	19.4	20.3	31.6	32.3	6.5	7.5	7.4	8.0
RIS1S241TX1	BR1	19.4	20.3	31.6	32.4	6.4	7.5	7.4	7.9
RIS1S269TX1	BRRF	19.4	20.3	31.6	32.4	6.5	7.5	7.5	8.0
Control	–	19.4	20.2	31.7	32.3	6.5	7.4	7.5	8.3

[1] Ambient salinity of Sequim Bay seawater during testing was about 32–33 ‰.

Table A-3. Reference Toxicant Test Results for the 10-day Solid Phase Test Using *Ampelisca abdita*, Site 16, Rhode Island Sound Sediments, November 2001.

Concentration	Units	Rep	Number Alive	Number Dead or Missing	Proportion Surviving	Proportion Surviving		
						Mean	Standard Deviation	Coefficient of Variation
0	mg/L Cd	1	20	0	1.00			
0	mg/L Cd	2	20	0	1.00			
0	mg/L Cd	3	18	2	0.90	0.97	0.06	6%
0.25	mg/L Cd	1	16	4	0.80			
0.25	mg/L Cd	2	16	4	0.80			
0.25	mg/L Cd	3	14	6	0.70	0.77	0.06	8%
0.5	mg/L Cd	1	13	7	0.65			
0.5	mg/L Cd	2	13	7	0.65			
0.5	mg/L Cd	3	12	8	0.60	0.63	0.03	5%
1	mg/L Cd	1	4	16	0.20			
1	mg/L Cd	2	10	10	0.50			
1	mg/L Cd	3	6	14	0.30	0.33	0.15	46%
2	mg/L Cd	1	0	20	0.00			
2	mg/L Cd	2	0	20	0.00			
2	mg/L Cd	3	0	20	0.00	0.00	0.00	NA
	LC_{50} Data:		*0.67 mg/L (95% Confidence Interval = 0.55-0.82 mg/L)*					
	Conc.		**0**	**0.25**	**0.5**	**1**	**2**	
	Total #		60	60	60	60	60	
	# Dead		2	14	22	40	60	

Table A-4. Results of the 10-day solid phase test using *Ampelisca abdita* to test coarse-grained sediments from Site 16, Rhode Island Sound, November 2001.

Station	Sample ID	Rep	Number Alive	Number Dead or Missing	Proportion Surviving	Mean Proportion Surviving	Standard Deviation	Coefficient of Variation
BR3	RIS1S20ATX1	1	19	1	0.95			
BR3	RIS1S20ATX1	2	19	1	0.95			
BR3	RIS1S20ATX1	3	19	1	0.95			
BR3	RIS1S20ATX1	4	20	0	1.00			
BR3	RIS1S20ATX1	5	18	2	0.90	0.95	0.04	4%
BR3	RIS1S20FTX1	1	19	1	0.95			
BR3	RIS1S20FTX1	2	20	0	1.00			
BR3	RIS1S20FTX1	3	20	0	1.00			
BR3	RIS1S20FTX1	4	20	0	1.00			
BR3	RIS1S20FTX1	5	19	1	0.95	0.98	0.03	3%
BR3	RIS1S212TX1	1	20	0	1.00			
BR3	RIS1S212TX1	2	15	5	0.75			
BR3	RIS1S212TX1	3	18	2	0.90			
BR3	RIS1S212TX1	4	19	1	0.95			
BR3	RIS1S212TX1	5	19	1	0.95	0.91	0.10	11%

APPENDIX B

10-day Solid Phase Test with *Eohaustorius estuarius*

Table B-1. Results of the 10-day solid phase test using *Eohaustorius estuarius*, Site 16, Rhode Island Sound sediments, November 2001.

Sample ID	Station ID	Rep	Number Alive	Number Dead or Missing	Proportion Surviving	Proportion Surviving		
						Mean	Standard Deviation	Coefficient of Variation
RIS1S1EDTX1	BR2	1	18	2	0.90			
RIS1S1EDTX1	BR2	2	17	3	0.85			
RIS1S1EDTX1	BR2	3	19	1	0.95			
RIS1S1EDTX1	BR2	4	19	1	0.95			
RIS1S1EDTX1	BR2	5	19	1	0.95	0.92	0.04	5%
RIS1S20ATX1	BR3	1	17	3	0.85			
RIS1S20ATX1	BR3	2	18	2	0.90			
RIS1S20ATX1	BR3	3	20	0	1.00			
RIS1S20ATX1	BR3	4	19	1	0.95			
RIS1S20ATX1	BR3	5	18	2	0.90	0.92	0.06	6%
RIS1S20FTX1	BR3	1	19	1	0.95			
RIS1S20FTX1	BR3	2	18	2	0.90			
RIS1S20FTX1	BR3	3	15	5	0.75			
RIS1S20FTX1	BR3	4	18	2	0.90			
RIS1S20FTX1	BR3	5	17	3	0.85	0.87	0.08	9%
RIS1S212TX1	BR3	1	19	1	0.95			
RIS1S212TX1	BR3	2	20	0	1.00			
RIS1S212TX1	BR3	3	19	1	0.95			
RIS1S212TX1	BR3	4	17	3	0.85			
RIS1S212TX1	BR3	5	17	3	0.85	0.92	0.07	7%
RIS1S269TX1 [1]	BRRF	1	20	0	1.00			
RIS1S269TX1	BRRF	2	15	5	0.75			
RIS1S269TX1	BRRF	3	19	1	0.95			
RIS1S269TX1	BRRF	4	19	1	0.95			
RIS1S269TX1	BRRF	5	19	1	0.95	0.92	0.10	11%
Control	–	1	20	0	1.00			
Control	–	2	19	1	0.95			
Control	–	3	18	2	0.90			
Control	–	4	19	1	0.95			
Control	–	5	19	1	0.95	0.95	0.04	4%

[1] Reference sediment.

Table B-2. Summary of Water Quality Measurements for the 10-day Solid Phase Test Using *Eohaustorius estuarius*, Site 16, Rhode Island Sound Sediments, November 2001.

Sample ID	Station ID	Temperature (° C)		Salinity (‰)		Dissolved Oxygen (mg/L)		pH (units)	
		Min	Max	Min	Max	Min	Max	Min	Max
	target range:	13.0	17.0	28.0	32.0	3.4	NA [1]	7.3	8.3
RIS1S1EDTX1	BR2	14.1	14.9	30.0	31.5	6.7	8.4	7.6	7.9
RIS1S20ATX1	BR3	14.1	14.9	29.9	31.4	6.8	8.4	7.6	7.8
RIS1S20FTX1	BR3	14.1	14.8	29.8	31.2	6.6	8.5	7.6	7.8
RIS1S212TX1	BR3	14.1	15.0	30.2	31.2	6.8	8.5	7.6	7.9
RIS1S269TX1	BRRF	14.1	15.0	30.0	31.4	5.8	8.5	7.6	7.9
Control	–	14.1	14.9	30.1	31.3	6.8	8.5	7.6	7.9

Table B-3. Reference toxicant test results for the 10-day solid phase test using *Eohaustorius estuarius*, Site 16, Rhode Island Sound sediments, November 2001.

Concentration	Units	Rep	Number Alive	Number Dead or Missing	Proportion Surviving	Proportion Surviving		
						Mean	Standard Deviation	Coefficient of Variation
0	mg/L Cd	1	15	5	0.75			
0	mg/L Cd	2	17	3	0.85			
0	mg/L Cd	3	15	5	0.75	0.78	0.06	7%
3.5	mg/L Cd	1	17	3	0.85			
3.5	mg/L Cd	2	18	2	0.90			
3.5	mg/L Cd	3	16	4	0.80	0.85	0.05	6%
7	mg/L Cd	1	17	3	0.85			
7	mg/L Cd	2	14	6	0.70			
7	mg/L Cd	3	7	13	0.35	0.63	0.26	41%
14	mg/L Cd	1	8	12	0.40			
14	mg/L Cd	2	4	16	0.20			
14	mg/L Cd	3	4	16	0.20	0.27	0.12	43%
28	mg/L Cd	1	0	20	0.00			
28	mg/L Cd	2	0	20	0.00			
28	mg/L Cd	3	0	20	0.00	0.00	0.00	0%
	LC_{50} Data:	11.2 mg/L (95% Confidence Interval = 10.1-12.5 mg/L)						
	Conc.		**0**	**3.5**	**7**	**14**	**28**	
	Total #		60	60	60	60	60	
	# Dead		13	9	22	44	60	