

APPENDIX F-5

Sediment Toxicity Testing Summary Report

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October, 2000



***LONG ISLAND SOUND
DREDGED MATERIAL DISPOSAL EIS***

**Sediment Toxicity Testing
Summary Report**

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1.0 INTRODUCTION

The U.S. Environmental Protection Agency (USEPA) Region 1, with assistance from the U.S. Army Corps of Engineers New England District (USACE-NED), has initiated the preparation of a comprehensive Environmental Impact Statement (EIS) in compliance with the National Environmental Policy Act (NEPA). The EIS will consider the potential designation of one or more dredged material disposal sites in the waters of Long Island Sound (LIS) in compliance with Section 102c of the Marine Protection Research and Sanctuaries Act (MPRSA) and 40 CFR 230.80 of EPA's regulations under Section 404 of the Clean Water Act.

An important part of the EIS is to evaluate the current potential toxicity of sediments at the existing dredged material disposal sites and at appropriate reference locations. To support the preparation of the EIS, sediments from 25 locations in Long Island Sound were collected in winter 2000. The objective of the sampling effort was to collect sediment samples for physical, chemical and toxicological evaluation, and for characterization of benthic community structure. The potential toxicity of these sediments was tested using 10-day amphipod bioassays.

The goal of testing sediment toxicity is to better understand the long-term fate and potential ecological implications of disposing contaminated dredged materials at each site. The toxicity tests provide a means of assessing potential direct impacts of the dredged materials to the health of benthic organisms living in and on the sediments. This toxicity information may be correlated with physical and chemistry characteristics to determine problematic sediment conditions or compared to the benthic community abundance and composition to help explain observed variability. This toxicity evaluation helps to develop site selection and impact criteria for each prospective candidate disposal site and refines the framework for potential future data collection and assessment analyses.

2.0 METHODS

2.1 COLLECTION OF SEDIMENT SAMPLES

To support the preparation of the EIS, field operations were conducted from 14 to 19 February 2000 to collect the requisite sample materials identified by the report, *Long Island Sound Data Review and Recommendations*, (ENSR 1999) prepared under Task Order 25 of Contract DACW33-96-D-004. Field sampling was conducted in conformance with the quality objectives and protocols specified in the Quality Assurance Protection Plan (QAPP) prepared for this project, ENSR Document 9000-184-303.

Marine sediments were collected from a total of 25 stations, collectively located in four recognized disposal sites in Long Island Sound (Figure 1), as listed below:

Western Long Island Sound (WLIS)	Central Long Island Sound (CLIS)
Cornfield Shoals Disposal Site (CSDS)	New London Disposal Site (NLDS)

Each of the four disposal sites was comprised of six sampling stations, with the exception of the CLIS site that contained seven; for a total of 25 locations. Sampling stations at each of the four sites were selected based on a review of local bathymetry, in conjunction with historical records, with the overall objective of obtaining materials representative of certain and distinct geographical areas of marine sediment in the proximate areas of each disposal site. These sampling stations were classified as "Active," "Historical," "No-Impact," or "Far-Field."

A complete summary of the sampling stations visited during the survey is presented in Document No. 9000184-2000-F02S,BC which delineates the station designation, the appropriate three character ID used for sample tracking purposes and the target latitude and longitude used for vessel positioning.

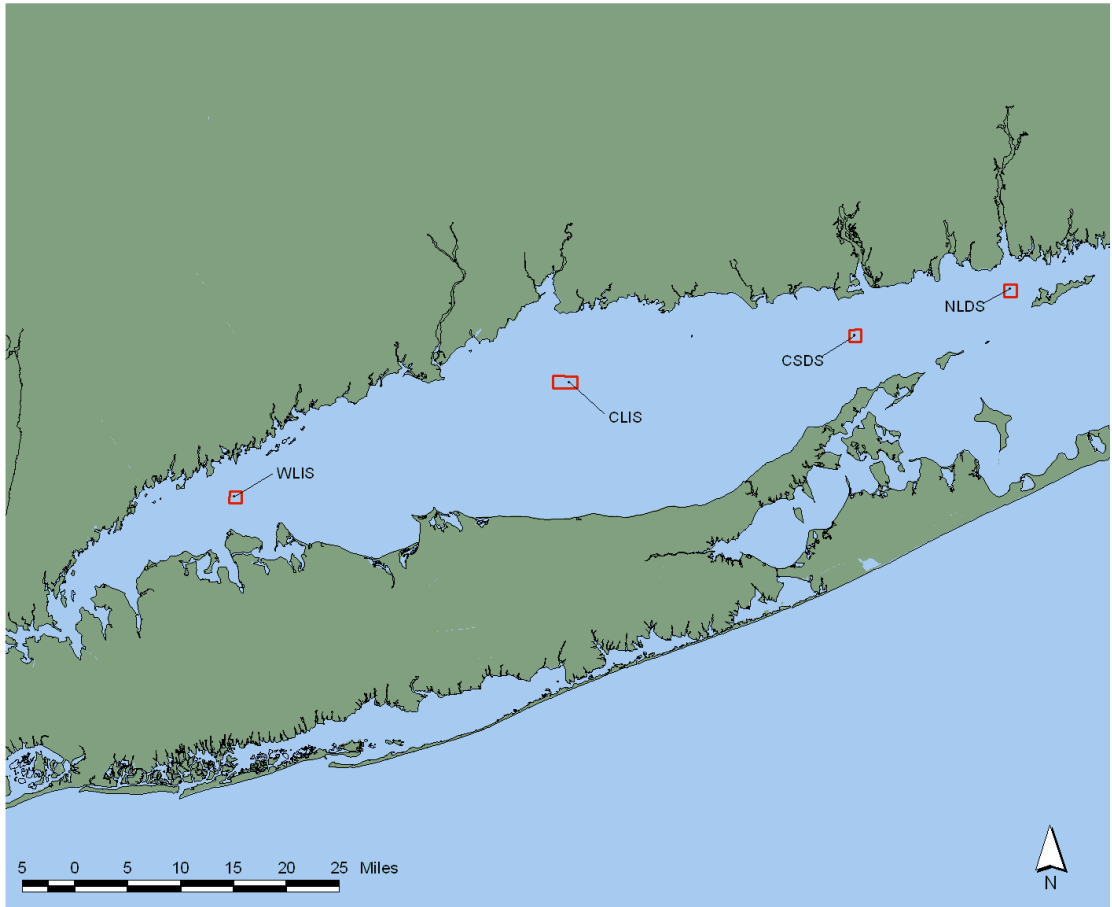
2.2 WHOLE SEDIMENT BIOASSAY TESTING PROGRAM

This section briefly describes the methods, equipment, and protocols used during the whole sediment testing program and summarizes the performance control measures. For additional detail regarding this testing program refer to Appendices A and B of this report.

2.2.1 Introduction

Bioassay tests were conducted at Springborn Laboratories, Inc. (SLI) located in Wareham, MA. Sediments were tested as 10-day solid phase acute exposure assays using the amphipod, *Ampelisca abdita*. The procedures used during the conduct of this study followed SLI Test Method SED-Aa-131b, "10-Day Toxicity Test with Marine Amphipods (*Ampelisca abdita*) to Meet U.S. EPA Guidelines". The

FIGURE 1 LONG ISLAND SOUND DISPOSAL SITE



procedures described in this method met the standard procedures described in EPA (1994) "*Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*," EPA 600-R-94-025, June 1994 and also ASTM Guideline E 1367-92, "Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods" (ASTM, 1997). The amphipod testing was conducted between March 1, 2000 and March 11, 2000. All tests were initiated within two weeks of the date of sediment collection.

The report from SLI containing results from the LIS whole sediment testing program is presented in Appendix A of this report. That report contains summarized test results, statistical comparisons, and reference toxicant test results. Additional information from the tests including daily observations, data bench sheets; water quality parameters (i.e., ammonia, dissolved oxygen, pH, and salinity) measured during the tests; and Chain-of-Custody (C-of-C) forms submitted with the samples are included in Appendix B. The text below provides an overview of the methods and results of the amphipod testing program.

2.2.2 Bioassay Material and Methods

Test Species Holding and Acclimation

The marine amphipod, *Ampelisca abdita*, was used to conduct the acute toxicity test. The marine amphipod, *A. abdita*, is widely distributed through out the East Coast of North America (Mills, 1964) and is considered a reasonable representative species of marine benthic invertebrates. The amphipod is easily collected and cultured, making it suitable for toxicity testing.

The amphipods were obtained from a commercial supplier (Eastern Aquatic Biosupply) and maintained in culture at SLI for a minimum of 7 days prior to testing. Upon receipt of the amphipods at SLI, organisms were acclimated to test temperature (20°C) and salinity (28‰). Temperature and salinity changes during the acclimation period did not exceed 3°C and 5‰ per 24 hour period. Once test temperature is reached, amphipods are held for a minimum of 2 days at test temperature and salinity.

During the holding and acclimation period prior to testing, the amphipods were fed a marine diatom, *Skeletonema sp.* (approximately 1.0×10^7 cells per mL), at a rate of 50 to 100 mL per day per culture vessel. During the 10-day toxicity test, the amphipods were not fed. Amphipods used to initiate the 10-day test are of approximately the same size and age (i.e., those that pass through a 1.0 mm sieve and are retained by a 0.7 mm sieve).

Sediment Preparation

Following field collection, sediment samples were shipped by ENSR overnight to SLI after collection (see Document No. 9000184-2000-F02S,BC regarding dates of collection and delivery). Upon receipt at SLI, sample containers were inspected for leakage or damage, the appropriate sample code or C-of-C identification recorded, and kept refrigerated at approximately $4 \pm 2^{\circ}\text{C}$ until testing.

Indigenous amphipod tubes were observed in the test samples received, therefore, prior to allocation of test sediments to their respective vessels, each sample was wet-pressed sieved through a 0.5 mm sieve to remove any live amphipods or organisms that would confound the results of the study. Due to their coarse nature, several samples (LIS01CS2KWCPG, LIS01CS4KWCPG, LISO1CSB92CPG, LIS01CSS94CPG) were wet-pressed through a 1.0 mm sieve and one sample (LIS01CSRF3CPG) was wet-pressed through a 0.71 mm sieve to remove any live amphipods or organisms

A laboratory control sediment is included in the toxicity test to evaluate performance of the test organisms and exposure system independent of potential contamination from the test sediments. A laboratory control sediment is collected from a site known to free of contamination and to provide acceptable conditions for survival of amphipods. SLI collected sediment from the Narrow River, Narragansett, Rhode Island (Lab Control - NR) and Little Harbor, Wareham, Massachusetts (Lab Control - LH) for use as the laboratory control sediments in this study. The sediment collected from Narrow River is representative of a fine silty sediment and the Little Harbor sediment is representative of a sandy sediment with a small amount of silt.

Test Apparatus and Conditions

The test vessels used in the bioassay were 1000 mL glass jars that are chemically cleaned. Test vessels are cleaned by an appropriate method to remove residue of test material previously used (i.e., acid to remove metals and bases; detergents and organic solvents to remove organic compounds) and rinsed several times using dilution water. Each jar contains approximately 2 cm of sediment (approximately 175 mL) and 800 mL of overlying water. Each test vessel has a 2 cm hole drilled approximately 13 cm from the bottom of the jar to allow for flow-through testing, if necessary. Each test vessel was labeled with the appropriate test concentration and replicate.

The test vessels are located in an area illuminated to a light intensity of 50 to 100 lux (46 to 93 footcandles) using a combination of fluorescent bulbs. Light intensity at the water surface is constantly maintained (24-hour light period).

Natural, filtered seawater from the Cape Cod Canal was used as dilution water for the test. Periodic analysis of representative samples of this seawater is conducted to ensure the absence of potential

toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the amphipods. The influent water is filtered through a 20- μm and 5- μm polypropylene core filter and adjusted to the required test temperature. Salinity and pH of each new batch of seawater is measured and recorded to ensure that these parameters are within acceptable ranges. Natural values for seawater are a pH range of 7.5 - 8.2 and salinity $32 \pm 3\%$. Salinity of the overlying water used in this test was adjusted to 28% prior to use.

Five replicates were maintained for each sediment sample, which consisted of 100% whole sediment sample (no dilutions). The laboratory control sediments were also conducted with five replicates. Twenty amphipods per replicate (100 organisms per sediment sample or control) were used to initiate the test.

The day before test initiation (Day -1), test sediment and control sediment are added to the replicate test vessels and the overlying water is added. The water is added gently to prevent suspension of the sediment layer in the water column. Test vessels are then covered, placed in a waterbath and gently aerated. This period allows the sediment and water to equilibrate prior to addition of the test organisms. During the allocation of each sediment sample to the replicate test vessels, a small sample (approximately 50 mL of pore water) will be generated from each sediment sample by centrifugation. Total ammonia, pH, salinity were measured in each pore water sample.

At test initiation (Day 0), amphipods are removed from the holding vessels. Twenty amphipods are impartially selected and distributed by pipetted into a replicate test or control vessel. This procedure is repeated until all vessels contain twenty amphipods. One hour after addition of the amphipods, each replicate vessel will be checked to ensure each amphipod has burrowed into the sediment. Those amphipods that have not burrowed into the substrate were replaced with amphipods from the same population.

Total ammonia was determined at test initiation and test termination (i.e., Day 0 and 10) in the overlying water from a composite sample from all replicates of each test and control sample. The composite sample is taken from a location of 1 to 2 cm above the sediment surface using a pipet. Dissolved oxygen, pH, temperature and salinity are measured in all replicate vessels at test initiation and test termination. In addition, dissolved oxygen, pH, temperature and salinity are monitored daily in one alternating replicate vessel during the course of the study. Temperature is monitored continuously in the water bath surrounding the exposure chambers using a minimum-maximum thermometer. Readings of temperature extremes are recorded daily.

Total dissolved oxygen is not allowed to drop below 60% of saturation for the duration of the study. Temperature of the overlying water is maintained at $20 \pm 1^\circ\text{C}$ by conducting the study in a temperature controlled waterbath maintained at the appropriate test temperature.

Total ammonia during the course of the tests (observation on Day 0 and Day 10) ranged from less than detection (<0.10 mg/l NH₃-N) to 1.4 mg/l. Since the total ammonia concentration at Day 0 was less than 20 mg/L, the samples were conducted as static exposure tests; i.e., the low ammonia levels meant that no flushing of the overlying seawater was required (Tudor T. Davies, 1993 Memorandum).

Survival of the amphipods was determined in each test vessel at test termination (Day 10) by sieving (0.5 mm sieve) the sediment to remove all surviving organisms. In addition, daily observations of organism behavior (e.g., sediment avoidance) and characteristics of test sediments and overlying water are also observed and recorded daily.

A summary of the 10-day whole sediment test conditions are provided in Table 1.

Performance Control

The test organism population was not to be used if >10% mortality was observed among the amphipods during the 48-hour period prior to test initiation. Acceptable mortality rates were observed during the initial holding and acclimation period.

A summary of the water quality characteristics of the sediment pore water and overlying water during the 10-day tests are presented in Tables 2, 3, and 4. Water quality remained acceptable throughout the 10-day exposure period. Throughout the exposure period, dissolved oxygen concentrations were generally greater than the required 60% of saturation (4.4 to 4.6 mg/L at 19 to 21°C). Ammonia concentrations, measured in pore water samples on Days -1 and 9 and in overlying water on Days 0 and 10 of the exposures, were <20 mg/L in all samples and were safely below toxicity levels. Water temperature, measured daily in the exposure vessels, ranged from 19 to 21°C.

The amphipod test is considered unacceptable if > 10% mortality occurred in the laboratory control population. The survivorship for the control populations was 99% for the NR sediment and 97% for the LH laboratory control sediments.

A 96-hour reference test, water-only exposure, using cadmium was conducted on a sub-population of organisms (from the same population used to initiate the test) within 30 days of the study. The health of the test population is assessed using the LC₅₀ from the reference test. The 96-hour reference test with *A. abdita* was initiated on March 2, 2000. The 96-hour LC₅₀ for cadmium for *A. abdita* was calculated to be 0.40 mg/L with confidence intervals of 0.34 to 0.48 mg/L. The reference test conducted on March 2, 2000 fell with the acceptable 2 standard deviation range established from the calculated LC₅₀ values.

TABLE 1
SUMMARY OF TEST CONDITIONS FOR SEDIMENT TOXICITY TEST
WITH *AMPELISCA ABDITA*

DATE SAMPLES RECEIVED:	February 18 to 21, 2000
TEST DATES:	March 1 to 11, 2000
TEST TYPE:	Whole-sediment toxicity test
TEMPERATURE:	19 to 21°C
LIGHT INTENSITY:	49 to 90 footcandles
PHOTOPERIOD:	24 hours light
TEST CHAMBER SIZE:	1000 mL
SEDIMENT VOLUME:	approximately 175 mL (2 cm layer)
OVERLYING WATER VOLUME:	800 mL
RENEWAL OF TEST SOLUTIONS:	None
SIZE TEST ORGANISMS:	Amphipods that passed through a 1.0 mm screen and were retained on a 0.7 mm screen
NUMBER OF ORGANISMS PER TEST CHAMBER:	20
NUMBER OF REPLICATE TEST CHAMBERS PER TREATMENT:	5
NUMBER ORGANISMS/SAMPLE:	100
FEEDING:	None
AERATION:	gentle oil-free aeration (2 to 3 bubble/minute)
TEST CONCENTRATION:	100% (no dilutions)
TEST DURATION:	10 days
ENDPOINTS:	Amphipod survival
TEST ACCEPTABILITY:	Minimum mean control survival of 90%

TABLE 2
SUMMARY OF SEDIMENT PORE WATER CHARACTERIZATION FOR THE
AMPELISCA ABDITA 10-DAY EXPOSURE

Sample Identification	pH		Salinity (‰)		Ammonia as N (mg/L)	
	Day -1	Day 9	Day -1	Day 9	Day -1	Day 9
Lab Control - NR	6.8	6.8	31	28	<0.10	6.9
Lab Control - LH	6.6	6.1	24	27	0.71	2.6
LIS01CL1KWCPG	7.1	6.9	27	28	<0.10	<0.10
LIS01CL2KWCPG	7.2	7.0	27	28	<0.10	<0.10
LIS01CL25WCPG	7.1	7.1	28	28	<0.10	<0.10
LIS01CLFVPCPG	7.2	7.1	28	29	<0.10	<0.10
LIS01CLN74CPG	7.4	7.5	28	28	<0.10	<0.10
LIS01CLN93CPG	7.1	6.8	30	29	0.86	<0.10
LIS01CLREFCPG	7.2	7.5	28	29	<0.10	<0.10
LIS01CS2KWCPG	7.6	7.6	25	28	<0.10	<0.10
LIS01CS4KWCPG	7.7	7.8	35	27	<0.10	<0.10
LIS01CSB92CPG	7.1	7.8	28	28	<0.10	<0.10
LIS01CSS94CPG	7.4	7.3	32	29	8.2	<0.10
LIS01CSRF4CPG	7.6	7.5	30	27	7.9	<0.10
LIS01CSRF3CPG	7.3	7.3	33	28	6.8	<0.10
LIS01NL1KECPG	7.1	6.9	32	28	12.4	<0.10
LIS01NL2KECPG	7.3	7.0	33	29	16.5	13.4
LIS01NLLRFCPG	7.2	7.3	34	28	10.7	12.2
LIS01NLRLLCCPG	7.0	7.0	31	29	15.6	4.4
LIS01NLSEACPG	7.1	6.9	32	30	13.1	5.7
LIS01NLWRFCPG	7.2	7.0	31	30	16.6	2.9
LIS01WLE5HCPG	7.1	7.0	30	28	<0.10	<0.10
LIS01WLW5HCPG	7.2	7.1	26	28	<0.10	<0.10
LIS01WLEB1CPG	6.9	7.0	30	29	<0.10	<0.10
LIS01WLMDICPG	7.2	7.2	26	28	<0.10	<0.10
LIS01WLSTHCPG	7.2	7.3	29	28	<0.10	<0.10
LIS01WLSWRCPG	7.2	7.2	27	28	<0.10	<0.10

TABLE 3
SUMMARY OF DISSOLVED OXYGEN, TEMPERATURE AND SALINITY RANGES IN THE
OVERLYING WATER OF THE *AMPELISCA ABDITA* 10-DAY EXPOSURE

Sample Identification	Dissolved Oxygen (mg/L)	Temperature (°C)	Salinity (‰)
	Day 0 - Day 10	Day 0 - Day 10	Day 0 - Day 10
Lab Control - NR	5.4 - 7.2	19 - 21	27 - 28
Lab Control - LH	5.8 - 6.7	19 - 21	26 - 28
LIS01CL1KWCPG	6.3 - 7.7	19 - 21	27 - 29
LIS01CL2KWCPG	6.1 - 7.7	19 - 21	27 - 29
LIS01CL25WCPG	5.9 - 7.3	19 - 21	27 - 28
LIS01CLFVPCPG	5.7 - 7.2	19 - 21	27 - 28
LIS01CLN74CPG	6.2 - 7.5	19 - 21	27 - 29
LIS01CLN93CPG	5.0 - 7.5	19 - 21	28 - 29
LIS01CLREFCPG	5.7 - 7.1	19 - 21	28 - 29
LIS01CS2KWCPG	6.3 - 7.5	19 - 21	27 - 30
LIS01CS4KWCPG	6.6 - 7.5	19 - 21	28
LIS01CSB92CPG	6.6 - 7.5	19 - 21	27 - 29
LIS01CSS94CPG	6.6 - 7.4	19 - 21	28 - 29
LIS01CSR4CPG	6.4 - 7.4	19 - 21	28 - 29
LIS01CSR3CPG	4.9 - 7.3	20 - 21	27 - 29
LIS01NL1KECPG	5.0 - 7.3	20 - 21	27 - 29
LIS01NL2KECPG	5.0 - 7.2	20 - 21	27 - 29
LIS01NLLRFCPG	4.9 - 7.4	20 - 21	27 - 29
LIS01NLRLLCCPG	5.0 - 7.2	20 - 21	28 - 29
LIS01NLSEACPG	5.0 - 7.2	20 - 21	28 - 30
LIS01NLWRFPCPG	5.0 - 7.2	20 - 21	28 - 30
LIS01WLE5HCPG	5.0 - 7.3	20 - 21	27 - 30
LIS01WLW5HCPG	4.9 - 7.1	20 - 21	27 - 29
LIS01WLEB1CPG	5.0 - 7.3	20 - 21	27 - 30
LIS01WLMDICPG	5.0 - 7.3	20 - 21	27 - 30
LIS01WLSTHCPG	5.0 - 7.2	20 - 21	27 - 30
LIS01WLSWRCPG	4.9 - 7.2	20 - 21	28 - 30

TABLE 4
SUMMARY OF pH AND AMMONIA MEASUREMENTS MADE IN THE OVERLYING WATER OF THE
AMPELISCA ABDITA 10-DAY EXPOSURE

Sample Identification	pH Range	Ammonia as N (mg/L)	
	Day 0 – Day 10	Day 0	Day 10
Lab Control - NR	7.5 - 8.0	0.46	<0.10
Lab Control - LH	7.3 - 7.9	0.16	1.4
LIS01CL1KWCPG	7.5 - 7.9	<0.10	<0.10
LIS01CL2KWCPG	7.6 - 8.0	<0.10	0.15
LIS01CL25WCPG	7.6 - 8.0	<0.10	<0.10
LIS01CLFVPCPG	7.7 - 8.3	<0.10	<0.10
LIS01CLN74CPG	7.6 - 7.9	<0.10	<0.10
LIS01CLN93CPG	7.7 - 8.2	<0.10	<0.10
LIS01CLREFCPG	7.7 - 8.1	<0.10	<0.10
LIS01CS2KWCPG	7.8 - 8.0	<0.10	<0.10
LIS01CS4KWCPG	7.8 - 8.0	<0.10	<0.10
LIS01CSB92CPG	7.7 - 8.0	<0.10	0.71
LIS01CSS94CPG	7.7 - 7.9	<0.10	1.3
LIS01CSR4CPG	7.7 - 7.9	<0.10	1.1
LIS01CSR3CPG	7.7 - 8.0	0.49	0.12
LIS01NL1KECPG	7.8 - 8.3	0.88	0.47
LIS01NL2KECPG	7.7 - 8.2	1.0	<0.10
LIS01NLLRCPG	7.8 - 8.2	1.0	0.59
LIS01NLRLCCPG	7.7 - 8.2	1.3	0.36
LIS01NLSEACPG	7.8 - 8.2	1.1	<0.10
LIS01NLWRFCPG	7.7 - 8.3	1.1	0.17
LIS01WLE5HCPG	7.7 - 8.5	0.22	<0.10
LIS01WLW5HCPG	7.8 - 8.2	<0.10	<0.10
LIS01WLEB1CPG	7.8 - 8.1	0.55	<0.10
LIS01WLMDICPG	7.7 - 8.0	0.43	<0.10
LIS01WLSTHCPG	7.7 - 8.0	0.23	<0.10
LIS01WLSWRCPG	7.7 - 8.0	0.22	<0.10

Data Analysis

The mean survival of organisms exposed in each test sediment and laboratory control sample was tested for normality (e.g., Shapiro-Wilks Test) and homogeneity of variance (e.g., Bartlett's Test). If the data set passed these two tests, then a parametric method (e.g., Dunnett's Test) was used to evaluate the results of the mean survival of each test sample for significant adverse effects versus the laboratory control and/or the reference control sample. If the data set failed the test for normality and homogeneity of variance, then a non-parametric method (e.g., Steel's Many-One Rank Test) was used to determine significant adverse effects. If necessary, mean survival values will be transformed (e.g., arcsine square).

Statistical comparison of all test samples was made versus both laboratory control samples. In addition, samples from each of the collection areas were statistically compared to the reference control sample from that area.

2.3 SUMMARY OF TEST RESULTS

The results from the toxicity testing from the 25 sediments are shown in Table 5. This table summarizes *A. abdita* survival at termination of the 10-day exposure period. The mean percent survival for organisms exposed to two laboratory control groups (Lab Control - NR and Lab Control - LH) was 99 and 97%, respectively, and met the minimum control performance survival criteria according to EPA guidelines (90%). In addition, the performance of the disposal site-specific reference control samples (LIS01CLREFCPG, LIS01CSR3CPG, LIS01NLLRF3CPG and LIS01WLSTHCPG) averaged 97, 98, 95 and 98% survival, respectively.

The mean percent survival in the sediment samples LIS01CS2KWCPG, LIS01CSB92CPG and LIS01NL1KECPG averaged 93, 87 and 92%, respectively and were statistically different compared to the survival of the Lab Control - NR organisms (i.e., 99%). The mean percent survival in the sediment sample LIS01CSB92CPG averaged 87% and was statistically different compared to the survival of the Lab Control - LH organisms (i.e., 99%) and the reference control LIS01CSR3CPG organisms (i.e., 98%). The mean percent survival in all other samples ranged from 94 to 100% and were comparable to either both Lab Control organisms or the respective reference control organisms.

The survivorship in sample LIS01CSB92CPG (87%) was slightly below the minimum control performance criteria (i.e., 90% survival). This result may be partially attributable to the coarse sandy nature of the sediment since reduced survival of *A. abdita* has been observed in sediments that are > 95% sand (U.S. EPA, 1994). However, other test sediments with >95% coarse sand substrate were not significantly less than controls. None of the sediments test had survivorship > 20% less than the laboratory control and site-specific reference locations. Therefore, it may be concluded that the sediment-associated constituents in these samples do not pose a toxic risk to benthic organisms.

TABLE 5
SUMMARY OF THE SURVIVAL OF *AMPELISCA ABDITA* AFTER 10 DAYS OF EXPOSURE

Sample Identification	Mean Percent Survival (Standard Deviation)
Lab Control - NR	99(2)
Lab Control - LH	97(4)
LIS01CLREFCPG (reference control)	97(3)
LIS01CL1KWCPG	98(3)
LIS01CL2KWCPG	98(3)
LIS01CL25WCPG	100(0)
LIS01CLFVPCPG	94(5)
LIS01CLN74CPG	100(0)
LIS01CLN93CPG	98(4)
LIS01CSRF3CPG (reference control)	98(3)
LIS01CS2KWCPG	93(6) ^a
LIS01CS4KWCPG	97(3)
LIS01CSB92CPG	87(4) ^{abc}
LIS01CSS94CPG	98(4)
LIS01CSRF4CPG	97(3)
LIS01NLLRFCPG (reference control)	95(0)
LIS01NL1KECPG	92(4) ^a
LIS01NL2KECPG	94(7)
LIS01NLRLLCCPG	98(3)
LIS01NLSEACPG	99(2)
LIS01NLWRFCPG	96(5)
LIS01WLSTHCPG (reference control)	98(3)
LIS01WLE5HCPG	96(2)
LIS01WLW5HCPG	98(3)
LIS01WLEB1CPG	98(3)
LIS01WLMDICPG	100(0)
LIS01WLSWRCPG	99(2)
^a Statistically different ($p < 0.05$) as compared to the laboratory control-NR survival (99%). ^b Statistically different ($p < 0.05$) as compared to the laboratory control-LH survival (97%). ^c Statistically different ($p < 0.05$) as compared to the reference control (LIS01CSRF3CPG) survival (99%)	

APPENDIX A

TOXICITY EVALUATION OF SEDIMENT WITH *AMPELISCA ABDITA*

SUMMARY REPORT

Study Title

Toxicity Evaluation of Sediment
with *Ampelisca abdita*

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EXECUTIVE SUMMARY

The following is a summary of testing performed with the marine amphipod (*Ampelisca abdita*) to evaluate sediment for ENSR, Survey LIS01. The twenty-five sediment test samples from Long Island Sound were identified as, collected by ENSR personnel and received at Springborn as outlined below:

Sample Identification	Date Sample was Collected	Date Received at Springborn
LIS01NL1KECPG	February 16, 2000	February 18, 2000
LIS01NL2KECPG	February 16, 2000	February 18, 2000
LIS01NLLRFCPG	February 16, 2000	February 18, 2000
LIS01CS2KWCPG	February 17, 2000	February 18, 2000
LIS01CS4KWCPG	February 17, 2000	February 18, 2000
LIS01CSS94CPG	February 17, 2000	February 18, 2000
LIS01CSRF4CPG	February 17, 2000	February 18, 2000
LIS01CSRF3CPG	February 17, 2000	February 18, 2000
LIS01NLRLCCPG	February 17, 2000	February 18, 2000
LIS01NLSEACPG	February 17, 2000	February 18, 2000
LIS01NLWRFCPG	February 17, 2000	February 18, 2000
LIS01CSB92CPG	February 18, 2000	February 18, 2000
LIS01WLE5HCPG	February 18, 2000	February 21, 2000
LIS01CL1KWCPG	February 19, 2000	February 21, 2000
LIS01CL2KWCPG	February 19, 2000	February 21, 2000
LIS01CL25WCPG	February 19, 2000	February 21, 2000
LIS01CLREFCPG	February 19, 2000	February 21, 2000
LIS01WLW5HCPG	February 19, 2000	February 21, 2000
LIS01WLEB1CPG	February 19, 2000	February 21, 2000
LIS01WLMDICPG	February 19, 2000	February 21, 2000
LIS01WLSTHCPG	February 19, 2000	February 21, 2000
LIS01WLSWRCPG	February 19, 2000	February 21, 2000
LIS01CLFVPCPG	February 20, 2000	February 21, 2000
LIS01CLN74CPG	February 20, 2000	February 21, 2000
LIS01CLN93CPG	February 20, 2000	February 21, 2000

In addition, Springborn collected sediment from the Narrow River, Narragansett, Rhode Island (Lab Control - NR) and Little Harbor, Wareham, Massachusetts (Lab Control - LH) for use as the laboratory control sediment in this study. The sediment collected from Narrow River was representative of a fine silty sediment and the Little Harbor sediment was representative of a sandy

sediment with a small amount of silt. All samples were tested in a single group. The samples were initiated on March 1, 2000 and terminated on March 11, 2000. All test were initiated within two weeks from the date of sample collection.

The procedures used during the conduct of this study followed Springborn Laboratories Test Method SED-Aa-131b, "10-Day Toxicity Test with Marine Amphipods (*Ampelisca abdita*) to Meet U.S. EPA Guidelines". The procedures described in this method met the standard procedures described in "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods" (U.S. EPA 1994) and also ASTM Guideline E 1367-92, "Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods" (ASTM, 1997). The test method followed during the conduct of this test is attached in Appendix I.

Indigenous amphipod tubes were observed in the test samples received, therefore, prior to allocation of test sediments to their respective vessels, each sample was wet-pressed sieved through a 0.5 mm sieve to remove any live amphipods or organisms that would confound the results of the study. Due to their coarse nature, several samples (LIS01CS2KWCPG, LIS01CS4KWCPG, LISO1CSB92CPG, LIS01CSS94CPG) were wet-pressed through a 1.0 mm sieve and one sample (LIS01CSRF3CPG) was wet-pressed through a 0.71 mm sieve to remove any live amphipods or ogranisms.

A 96-hour reference test with *A. abdita* was initiated on March 2, 2000. Cadmium chloride was the reference toxicant used. The organisms used to initiate the reference test were from the same population of organisms used to initiate all of test sediments. The 96-hour LC50 for cadmium and *A. abdita* was calculated to be 0.40 mg/L with confidence intervals of 0.34 to 0.48 mg/L. Appendix II contains the control chart for this reference test and previously conducted reference tests. The reference test conducted in March 2, 2000 fell with the acceptable 2 standard deviation range established from the calculated LC50 values.

A summary of the water quality characteristics of the sediment pore water and overlying water during the 10-day tests are presented in Tables 1, 2 and 3. Water quality remained acceptable throughout the 10-day exposure period. Throughout the exposure period, dissolved oxygen concentrations were generally greater than the required 60% of saturation (4.4 to 4.6 mg/L at 19 to 21°C). Ammonia concentrations, measured in pore water samples on days -1 and 9 and in overlying water on days 0 and 10 of the exposures, were ≤ 20 mg/L in all samples and were safely below toxicity levels. Water temperature, measured daily in the exposure vessels, ranged from 19 to 21 °C.

A summary of the *Ampelisca abdita* survival at termination of the 10-day exposure period is presented in Table 4. The mean percent survival for organisms exposed to two laboratory control groups (Lab Control - NR and Lab Control - LH) was 99 and 97%, respectively, and met the minimum control performance survival criteria according to EPA guidelines (90%). In addition, the performance of the reference control samples (LIS01CLREFCPG, LIS01CSRF3CPG, LIS01NLLRFPG and LIS01WLSTHCPG) averaged 97, 98, 95 and 98% survival, respectively.

Statistical comparison of all test samples was made versus both laboratory control samples. In addition, samples from each of the for collection areas were statistically compared to the reference control sample from that area.

The mean percent survival in the sediment samples LIS01CS2KWCPG, LIS01CSB92CPG and LIS01NL1KECPG averaged 93, 87 and 92%, respectively and were statistically different compared to the survival of the Lab Control - NR organisms (i.e., 99%). The mean percent survival in the sediment sample LIS01CSB92CPG averaged 87% and was statistically different compared to the survival of the Lab Control - LH organisms (i.e., 99%) and the reference control LIS01CSRF3CPG organisms (i.e., 98%). The mean percent survival in all other samples ranged from 94 to 100% and were comparable to either both Lab Control organisms or the respective reference control organisms.

CONCLUSIONS

Results of the samples tested established that the laboratory control organism and all the test samples, except sample LIS01CSB92CPG, met the minimum control performance criteria for this type of study (i.e., 90% survival). The survival observed in sample LIS01CSB92CPG (87%) was slightly below the minimum criteria and could be attributed to the coarse sandy nature of the sediment. Reduced survival of *A. abdita* has been observed in sediments that are > 95% sand (U.S. EPA, 1994). Therefore, the results of the survival effects observed in these samples suggest the absence of adverse effects associated with all of the samples tested.

SUMMARY OF TEST CONDITIONS
10-Day Sediment Toxicity Test with *Ampelisca abdita*

DATE SAMPLES RECEIVED: February 18 to 21, 2000

TEST DATES: March 1 to 11, 2000

TEST TYPE: Whole-sediment toxicity test

TEMPERATURE: 19 to 21°C

LIGHT INTENSITY: 49 to 90 footcandles

PHOTOPERIOD: 24 hours light

TEST CHAMBER SIZE: 1000 mL

SEDIMENT VOLUME: approximately 175 mL (2 cm layer)

OVERLYING WATER VOLUME: 800 mL

RENEWAL OF TEST SOLUTIONS: None

SIZE TEST ORGANISMS: Amphipods that passed through a 1.0 mm screen and were retained on a 0.7 mm screen

NUMBER OF ORGANISMS PER TEST CHAMBER: 20

NUMBER OF REPLICATE TEST CHAMBERS PER TREATMENT: 5

NUMBER ORGANISMS/SAMPLE: 100

FEEDING: None

AERATION: gentle oil-free aeration (2 to 3 bubble/minute)

TEST CONCENTRATION: 100% (no dilutions)

TEST DURATION: 10 days

ENDPOINTS: Amphipod survival

TEST ACCEPTABILITY: Minimum mean control survival of 90%

Table 1. Summary of sediment pore water characterization for the *Ampelisca abdita* 10-day exposure.

Sample Identification	pH		Salinity (‰)		Ammonia as N (mg/L)	
	Day -1	Day 9	Day -1	Day 9	Day -1	Day 9
Lab Control - NR	6.8	6.8	31	28	<0.10	6.9
Lab Control - LH	6.6	6.1	24	27	0.71	2.6
LIS01CL1KWCPG	7.1	6.9	27	28	<0.10	<0.10
LIS01CL2KWCPG	7.2	7.0	27	28	<0.10	<0.10
LIS01CL25WCPG	7.1	7.1	28	28	<0.10	<0.10
LIS01CLFVPCPG	7.2	7.1	28	29	<0.10	<0.10
LIS01CLN74CPG	7.4	7.5	28	28	<0.10	<0.10
LIS01CLN93CPG	7.1	6.8	30	29	0.86	<0.10
LIS01CLREFCPG	7.2	7.5	28	29	<0.10	<0.10
LIS01CS2KWCPG	7.6	7.6	25	28	<0.10	<0.10
LIS01CS4KWCPG	7.7	7.8	35	27	<0.10	<0.10
LIS01CSB92CPG	7.1	7.8	28	28	<0.10	<0.10
LIS01CSS94CPG	7.4	7.3	32	29	8.2	<0.10
LIS01CSR4CPG	7.6	7.5	30	27	7.9	<0.10
LIS01CSR3CPG	7.3	7.3	33	28	6.8	<0.10
LIS01NL1KECPG	7.1	6.9	32	28	12.4	<0.10
LIS01NL2KECPG	7.3	7.0	33	29	16.5	13.4
LIS01NLLRFPCPG	7.2	7.3	34	28	10.7	12.2
LIS01NLRLCCPG	7.0	7.0	31	29	15.6	4.4
LIS01NLSEACPG	7.1	6.9	32	30	13.1	5.7
LIS01NLWRFCPG	7.2	7.0	31	30	16.6	2.9
LIS01WLE5HCPG	7.1	7.0	30	28	<0.10	<0.10
LIS01WLW5HCPG	7.2	7.1	26	28	<0.10	<0.10
LIS01WLEB1CPG	6.9	7.0	30	29	<0.10	<0.10
LIS01WLMDICPG	7.2	7.2	26	28	<0.10	<0.10
LIS01WLSTHCPG	7.2	7.3	29	28	<0.10	<0.10
LIS01WLSWRCPG	7.2	7.2	27	28	<0.10	<0.10

Table 2. Summary of dissolved oxygen, temperature and salinity ranges in the overlying water of the *Ampelisca abdita* 10-day exposure.

Sample Identification	Dissolved Oxygen (mg/L)	Temperature (°C)	Salinity (‰)
	Day 0 - Day 10	Day 0 - Day 10	Day 0 - Day 10
Lab Control - NR	5.4 - 7.2	19 - 21	27 - 28
Lab Control - LH	5.8 - 6.7	19 - 21	26 - 28
LIS01CL1KWCPG	6.3 - 7.7	19 - 21	27 - 29
LIS01CL2KWCPG	6.1 - 7.7	19 - 21	27 - 29
LIS01CL25WCPG	5.9 - 7.3	19 - 21	27 - 28
LIS01CLFVPCPG	5.7 - 7.2	19 - 21	27 - 28
LIS01CLN74CPG	6.2 - 7.5	19 - 21	27 - 29
LIS01CLN93CPG	5.0 - 7.5	19 - 21	28 - 29
LIS01CLREFCPG	5.7 - 7.1	19 - 21	28 - 29
LIS01CS2KWCPG	6.3 - 7.5	19 - 21	27 - 30
LIS01CS4KWCPG	6.6 - 7.5	19 - 21	28
LIS01CSB92CPG	6.6 - 7.5	19 - 21	27 - 29
LIS01CSS94CPG	6.6 - 7.4	19 - 21	28 - 29
LIS01CSRF4CPG	6.4 - 7.4	19 - 21	28 - 29
LIS01CSRF3CPG	4.9 - 7.3	20 - 21	27 - 29
LIS01NL1KECPG	5.0 - 7.3	20 - 21	27 - 29
LIS01NL2KECPG	5.0 - 7.2	20 - 21	27 - 29
LIS01NLLRFCPG	4.9 - 7.4	20 - 21	27 - 29
LIS01NLRLCCPG	5.0 - 7.2	20 - 21	28 - 29
LIS01NLSEACPG	5.0 - 7.2	20 - 21	28 - 30
LIS01NLWRFCPG	5.0 - 7.2	20 - 21	28 - 30
LIS01WLE5HCPG	5.0 - 7.3	20 - 21	27 - 30
LIS01WLW5HCPG	4.9 - 7.1	20 - 21	27 - 29
LIS01WLEB1CPG	5.0 - 7.3	20 - 21	27 - 30
LIS01WLMDICPG	5.0 - 7.3	20 - 21	27 - 30
LIS01WLSTHCPG	5.0 - 7.2	20 - 21	27 - 30
LIS01WLSWRCPG	4.9 - 7.2	20 - 21	28 - 30

Table 3. Summary of pH and ammonia measurements made in the overlying water of the *Ampelisca abdita* 10-day exposure.

Sample Identification	pH Range Day 0 - Day 10	Ammonia as N (mg/L)	
		Day 0	Day 10
Lab Control - NR	7.5 - 8.0	0.46	<0.10
Lab Control - LH	7.3 - 7.9	0.16	1.4
LIS01CL1KWCPG	7.5 - 7.9	<0.10	<0.10
LIS01CL2KWCPG	7.6 - 8.0	<0.10	0.15
LIS01CL25WCPG	7.6 - 8.0	<0.10	<0.10
LIS01CLFVPCPG	7.7 - 8.3	<0.10	<0.10
LIS01CLN74CPG	7.6 - 7.9	<0.10	<0.10
LIS01CLN93CPG	7.7 - 8.2	<0.10	<0.10
LIS01CLREFCPG	7.7 - 8.1	<0.10	<0.10
LIS01CS2KWCPG	7.8 - 8.0	<0.10	<0.10
LIS01CS4KWCPG	7.8 - 8.0	<0.10	<0.10
LIS01CSB92CPG	7.7 - 8.0	<0.10	0.71
LIS01CSS94CPG	7.7 - 7.9	<0.10	1.3
LIS01CSR4CPG	7.7 - 7.9	<0.10	1.1
LIS01CSR3CPG	7.7 - 8.0	0.49	0.12
LIS01NL1KECPG	7.8 - 8.3	0.88	0.47
LIS01NL2KECPG	7.7 - 8.2	1.0	<0.10
LIS01NLLRFPCPG	7.8 - 8.2	1.0	0.59
LIS01NLRLCCPG	7.7 - 8.2	1.3	0.36
LIS01NLSEACPG	7.8 - 8.2	1.1	<0.10
LIS01NLWRFCPG	7.7 - 8.3	1.1	0.17
LIS01WLE5HCPG	7.7 - 8.5	0.22	<0.10
LIS01WLW5HCPG	7.8 - 8.2	<0.10	<0.10
LIS01WLEB1CPG	7.8 - 8.1	0.55	<0.10
LIS01WLMDICPG	7.7 - 8.0	0.43	<0.10
LIS01WLSTHCPG	7.7 - 8.0	0.23	<0.10
LIS01WLSWRCPG	7.7 - 8.0	0.22	<0.10

Table 4. Summary of the survival of *Ampelisca abdita* after 10 days of exposure .

Sample Identification	Mean Percent Survival (Standard Deviation)
Lab Control - NR	99(2)
Lab Control - LH	97(4)
LIS01CLREFCPG (reference control)	97(3)
LIS01CL1KWCPG	98(3)
LIS01CL2KWCPG	98(3)
LIS01CL25WCPG	100(0)
LIS01CLFVPCPG	94(5)
LIS01CLN74CPG	100(0)
LIS01CLN93CPG	98(4)
LIS01CSR3CPG (reference control)	98(3)
LIS01CS2KWCPG	93(6) ^a
LIS01CS4KWCPG	97(3)
LIS01CSB92CPG	87(4) ^{abc}
LIS01CSS94CPG	98(4)
LIS01CSR4CPG	97(3)
LIS01NLLRF3CPG (reference control)	95(0)
LIS01NL1KECPG	92(4) ^a
LIS01NL2KECPG	94(7)
LIS01NLRLCCPG	98(3)
LIS01NLSEACPG	99(2)
LIS01NLWRFCPG	96(5)
LIS01WLSTHCPG (reference control)	98(3)
LIS01WLE5HCPG	96(2)
LIS01WLW5HCPG	98(3)
LIS01WLEB1CPG	98(3)
LIS01WLMDICPG	100(0)
LIS01WLSWRCPG	99(2)

^a Statistically different ($p \leq 0.05$) as compared to the laboratory control-NR survival (99%).

^b Statistically different ($p \leq 0.05$) as compared to the laboratory control-LH survival (97%).

^c Statistically different ($p \leq 0.05$) as compared to the reference control (LIS01CSR3CPG) survival (99%).

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