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THE APPLICATION OF THE AMPHIPOD TEN-DAY SEDIMENT TOXICITY TEST FOR DREDGED MATERIAL EVALUATIONS

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INTRODUCTION

The Environmental Protection Agency and Army Corps of Engineers is in the process of revising the current EPA/COE Implementation Manual (1977) for the evaluation of dredged materials for ocean disposal. The revised document will recommend that acute toxicity tests utilize sediment-dwelling amphipods as the species of choice. This recommendation is based primarily on the experience of Swartz (1985) and others on the U.S. west coast, who have developed an extensive database with the phoxocephalid amphipod Rhepoxynius abronius. In a complementary effort, the Environmental Research Laboratory-Narragansett has been developing and applying similar test procedures using the ampeliscid amphipod Ampelisca abdita. This is the only amphipod for which there are sediment toxicity data on east coast sediments, and these are restricted to sites in New England (e.g., Black Rock Harbor, New Haven Harbor, New Bedford Harbor, and Narragansett Bay, among others). This also is one of the few amphipods that has been employed in full life cycle toxicity tests with sediments (Scott and Redmond 1989).

Before the U.S. Army Corps of Engineer District Offices begin to implement the recommended procedures, it will be important to establish the relative sensitivity of the test species and the effects of sediment grain size on sensitivity, and to develop protocols for interpreting acute toxicity and emergence data. Specific guidance on the most compatible species for sediments of varying grain sizes will also be required.

This paper describes the application of sediment toxicity test procedures using two amphipods available on the U.S. east coast, Ampelisca abdita and Rhepoxynius hudsoni. The latter species is a congener of R. abronius. It appears to have the same habitat requirements as the west coast amphipod, and according to some preliminary testing at the ERL-Narragansett, exhibits a range of sensitivity similar to that of Ampelisca.

Ampelisca abdita is a euryhaline benthic amphipod which ranges from Maine to south-central Florida and the eastern Gulf of Mexico. It is found in fine sediments from the intertidal zone to depths of 60 meters. This amphipod constructs a soft, upright, membranous tube 3 to 4 cm long in surface sediments. A. abdita is a particle feeder, ingesting either surface-deposited particles or particles in suspension. Where large communities of Ampelisca are present, patches of amphipod tubes may affect benthic community structure by creating a topographically complex microhabitat and by trapping suspended material. This amphipod is also a common food source for bottom fish, such as flounder (Richards 1963).

Acute and chronic test methods have been developed using this species to evaluate the biological effects of contaminated sediments. The acute method has been applied to test sediments from Black Rock Harbor, CT (Rogerson et al. 1985), New Haven Harbor, CT, Bridgeport Harbor, CT, New Bedford Harbor, MA, and San Francisco Bay (Bretler et al. 1989, Long and Buchman 1989). The chronic test method has been applied to the evaluation of Black Rock Harbor sediments in the suspended phase (Scott and Redmond 1989) but, to date, has not been used in bedded sediment exposures.

Rhepoxynius hudsoni (formerly Trichophoxus epistomus) is a free burrowing phoxocephalid amphipod inhabiting medium to fine sands from the shallow subtidal to depths greater than 50 m. It is found in coastal waters from southern Maine to North Carolina (Bousfield 1973). This species has an annual life cycle with ovigerous females present from May to September. Little else is known about its biology; however, its congener, R. abronius, is a predator on interstitial fauna (Oliver et al. 1982).

R. abronius has been used extensively on the west coast to evaluate contaminated sediments and this methodology has received broad acceptance in the regulatory community. It has been demonstrated that Rhepoxynius and Ampelisca prefer opposite ends of the grain size spectrum, with the former inhabiting coarser sediments (Swartz et al. 1985) and the latter inhabiting finer sediments (Mills 1967). Although there may be some restrictions on the use of the two phoxocephalid species due to effects of grain size (Spies 1989), it would be appropriate to determine the utility of an R. hudsoni test with sediments typical of those found in the mid-Atlantic Bight region.

The objectives of this study were to:

* determine the toxicity of ten sediments exhibiting a range of contamination and sediment particle size to the two amphipod species,

* examine the relationship between toxicity, contaminant concentrations and particle size, and

* determine the utility of the amphipod ten-day test for dredged material evaluations.

METHODS

SEDIMENT COLLECTION AND HOLDING

Sediment samples were collected from ten stations in the New

York-New Jersey Harbor area and at two additional sites designated as Reference and Control sites. The latter two stations are located east of Sandy Hook, NJ, and in Western Long Island Sound, respectively. Station locations are given in Table 1.

Using a Smith-McIntyre sediment sampler, a minimum of three grabs from each station were composited on deck in a pre-cleaned polyethylene tub using a pre-cleaned teflon scoop or spoon. Excess water in the grab was decanted in a manner that avoided loss of sediment. After mixing, the composited sediment samples were placed in pre-cleaned one gallon glass jars with Teflon-lined caps.

One gallon glass jars, lids, and teflon liners were detergent washed, and rinsed with deionized water. Jars were then acid-stripped and combusted at 450°C and stored capped until use. The grab sampler and other collection equipment were rinsed with seawater and/or deionized water, methanol, and methylene chloride between samples. Collection of enough sediment to fill at least three fourths (3/4) of the jar provided enough sample for all analyses including quality control analyses. Sufficient room was left in the jar to allow for mixing of the sample.

Samples were stored in the dark at 4°C until homogenization and subsampling for the toxicity tests and chemistry analyses. The toxicity tests and chemistry subsampling were conducted within ten days of sample collection. Chemistry samples were stored frozen until extraction. Adequate space was left in the jar for expansion of the frozen sample. Organic extracts were analyzed within 30 days of extraction to limit loss of the more labile analytes. These extracts were refrigerated at 4°C prior to analysis.

An empty sample jar was carried through all processing and handling steps to serve as a field blank. A solvent rinse of the jar was analyzed for organic contaminants along with the sample batch as a check on contamination that may have occurred during collection, shipping and/or storage.

PHYSICAL CHARACTERIZATION

Sediments were analyzed for their basic properties (e.g. grain size distribution, mode, mean grain size, median grain size, water content) using pipette analysis for the silt and clay fraction, sieving techniques for the sand fraction and methods of moments calculations (Folk 1968). Each site was then characterized on the basis of sand, silt and clay percentages as determined by these analyses (Folk 1970).

Grain size results are reported as either percentages or in phi

units. Critical boundaries are the sand/silt boundary at 4 phi (0.063 mm) and the silt/clay boundary at 8 phi (0.004 mm). The conversion from millimeters to phi units is accomplished using:

$$\text{phi} = -\log_2 (\text{grain size in mm})$$

Silt/Clay Content

An aliquot of the sediments at each station was taken so as to yield 9-15 g of sediment in the fine sand size fraction (<0.063 mm). Samples were placed in 50 mL beakers in a solution of deionized water and 10 mL (0.051 g) of dispersant and allowed to remained until they were disaggregated. A rubber tipped stirring rod or spatula was used to further break up the sample. The disaggregated sample was wet-sieved through a 4 phi (0.063 mm) sieve using deionized water. The fine material (i.e. silts and clays) was collected in an underlying evaporating dish.

The material on the sieve (i.e. >0.063 mm fraction) was transferred to another beaker for use in the determination of sand content. This material was oven dried at 100°C and weighed to the nearest 0.001 g.

The silt/clay fraction which was collected in the evaporating dish was transferred to a graduated one liter glass column. The column was filled with deionized water to the 1000 mL mark, thoroughly mixed using a glass stirring rod and stored for 12 hours. If flocculation of the clay fraction was observed, the previous steps were repeated using more sample volume.

After approximately 12 hours, the column was thoroughly stirred and a pipette with depth gradations was used to withdraw 20 mL aliquots at the specified time and depth intervals. Withdrawal times were determined based on water temperature, measured by thermometer, at the beginning of the analysis. The coarse to fine silt fraction (4-7 phi, 0.062-0.008 mm particle diameter) of these sediments was the most intensively sampled, with withdrawal times scheduled to obtain samples at particle size intervals of 0.5 phi units (~ 0.010 mm). The very fine-clay fraction (7-9 phi, 0.008-0.002 particle diameter) was sampled at whole phi intervals corresponding to particle size intervals of approximately 0.020 mm.

The pipetted material was placed into individual beakers and oven-dried at 100-130°C for approximately 24 hours. After drying, the beakers were removed from the oven and left to cool for at least 1.5 hours allowing the clay fraction to equilibrate with the humidity in the room. The beakers and their contents were weighed to the nearest 0.001 g.

The weight of the sediment from the 4 phi sample was multiplied

by 50 to relate the subsample size to that of the whole sample and the weight of the dispersant was subtracted to give the total weight of the mud (F). This value was then added to the weight of the sand (S; see above) to give the total weight of the sample. The quantity obtained by multiplying each later pipette sample (4.5 phi, 5.0 phi, etc.) by 50 is (P). Cumulative weight percentages were then determined by substituting the above values into the following formula (Folk 1968):

$$\text{Cumulative Percent Coarser} = 100\,(S+F-P)/\,S+F$$

where S=weight of sand
F=weight of mud (silt + clay)
P=weight of each size fraction

Sand Content

The sand fraction was determined using sieving procedures. The greater than 4 phi fraction (>0.063 mm) was processed and dried during the separation of the sand fraction from the mud fraction for pipette analysis. This material was then poured through 8 inch diameter screens nested according to decreasing diameter and increasing phi size. The sieves were arranged at 0.5 phi (~0.010 mm) intervals between -0.5 phi (1.4 mm) and 4.0 phi (0.063 mm).

The screens were placed in a Ro-Tap shaker device for 15 minutes. The material from each sieve or size fraction was placed in a tared pan and weighed. Each fraction was then examined under a binocular microscope to estimate the percentage of aggregates in the sample. This was done by counting 100 grains and subtracting the aggregate proportion from the sample weight.

Cumulative percent was determined by dividing the total cumulative weight by the weight of each size fraction times 100. These values were then plotted on a cumulative curve.

Water Content

Water content (W), the amount of water present in a porous sediment, was determined using the formula:

$$W = 100(\text{wet wt.} - \text{dry wt.})/\text{wet wt.}$$

where wet wt.= weight of a water-saturated sample
dry wt.= weight of an oven-dried (24 hr at 100°C).

Other Sediment Parameters

Method of moments calculations were used to determine mean grain

size, sorting (the range of particle-sizes on either side of an average in sediments), skewness (a measure of asymmetry of the frequency of grain-size distribution) and kurtosis (the condition of peakedness of the grain-size distribution curve). The method of moments is a statistical technique that uses the individual weight percentages of the various size classes to determine the above variables. The major advantages of using this technique are the speed of analysis (in comparison to graphic analytical procedures) and freedom from the fundamental assumption that the sediments have lognormal size distributions (Blatt et al. 1972). Median grain sizes were determined from cumulative frequency curves generated by the grain size analysis.

The mean was calculated as the first moment and expressed as:

$$\bar{X} = \sum x/N$$

where $\bar{X}$ = mean grain diameter
x = grain diameter * frequency of occurrence (weight %)
N = number of measurements (100 when dealing with percents; Lewis 1984).

The second moment was the variance and expressed as:

$$m_2 = \sum (x-\bar{X})^2/ N-1$$

from which the standard deviation (i.e. the sorting) can be calculated as:

$$\sigma = \text{square root } m_2.$$

The third moment is:

$$m_3 = \sum (x-\bar{X})^3/N-1.$$

The skewness is calculated by:

$$\text{Skewness} = m_3/m_2^{(3/2)}.$$

The fourth moment is:

$$m_4 = \sum (x-\bar{X})^4/N-1.$$

The kurtosis is calculated by:

$$\text{Kurtosis} = m_4/m_2^2$$

CHEMICAL CHARACTERIZATION

Organic Compounds

Analytical Procedures

All glassware used in sediment sample analyses was detergent washed and rinsed with tap and deionized water according to ERLN Standard Operating Procedures (SOP). The glassware was then capped with aluminum foil and placed into a muffle furnace at 450°C for 6 hours. They were then stored capped with foil until used, at which time it was solvent-rinsed.

Cross-contamination between samples was minimized in all steps of the analysis by the use of clean glassware; clean pipettes for transferring samples; and by rinsing syringes between samples with solvent. Because carryover can occur when high- and low-level standards or samples are analyzed sequentially, high-level standards or samples were followed by an appropriate solvent blank to eliminate carryover.

All samples were prepared for analyses following ERLN SOPs.

PCBs, Pesticides, and PAHs: Prior to the removal of a sediment aliquot for analysis, the sediment and any standing water were physically stirred to create a homogeneous mixture. Non-representative material (e.g., rocks, shells, leaves, and twigs) present in the sample was discarded. A 3-5 g aliquot was weighed into a tared aluminum pan and oven-dried at 90°C for 24 h. The resulting dry weight was used to calculate a dry/wet ratio. These weights were manually recorded as they were taken.

Sediment samples were weighed into solvent-rinsed centrifuge tubes and a specified amount of internal standard was added. Contaminants of interest were initially extracted in acetonitrile using a sonic probe. The solvent/sediment mixture was centrifuged and the resulting solvent layer was poured into a separatory funnel containing pentane-extracted deionized water. The resultant mixture was further extracted using pentane and the extract was dried using anhydrous sodium sulfate.

Chemical class separations were achieved by silica gel column chromatography. One mL of pentane extract was charged onto a glass column containing 7.5% deactivated silica gel. Two fractions were collected using different solvent systems. PCBs were collected in the first fraction using pentane as the solvent system. The second fraction, eluted with 20% methylene chloride:pentane, contained polynuclear aromatic hydrocarbons (PAHs) and pesticides. Fractions were volume reduced to 1 mL and solvent exchanged to heptane before gas chromatographic analysis.

Quantification of PCBs and pesticides was accomplished using a Hewlett-Packard 5890 GC-ECD interfaced to a Perkin Elmer LIMS 2000 computer system by the Chromatography Laboratory Automation System. Identification and quantitation of analytes were performed via automated computer programs and checked by the

operator. Confirmations were made by GC-MS and/or by analysis of standards if unusual chromatographic patterns were present.

Quantification of PAHs was accomplished using a Finnigan MAT 4531 gas chromatograph - mass spectrometer (GC-MS) using electron impact ionization at 70 eV. Samples were injected onto a 30 meter DB-5 fused silica capillary column in the GC while the MS was scanning the range of 15 to 650 atomic mass units (amu) per second.

Dioxins and Furans: Sediment samples were homogenized as above. A small portion was weighed out for dry/wet determination. The samples were then mixed with sodium sulfate and allowed to dry. After drying, the appropriate standards were added and the sample was Soxhlet extracted. The extracts were volume reduced and the 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) were separated from most other interfering compounds in the extracts using several fractionation steps. These procedures included column chromatography on acid and base treated silica gels, silver nitrate impregnated silica gel, neutral alumina, and a silica gel/carbon mixture and are described in detail in the ERLN dioxin SOP.

As with PAHs, quantification of TCDD and TCDF was accomplished using the Finnigan MAT 4531. The MS was operated in the multiple ion detection (MID) mode, scanning ions 257, 304, 306, 320, 322, 328, and 334 which represented parent and confirmation peaks. Both compounds were quantified against the appropriate labeled surrogate compounds that were spiked into the samples at the beginning of the extraction. The recovery of these surrogates was also measured against external standards that were added at the time of injection. Detection limits for TCDD and TCDF were in the low parts per trillion range.

Instrument Calibration and Maintenance

Gas chromatographic methods used for quantifying pesticides and PCB congeners were calibrated using a single concentration standard. Standards were placed before and after the sample set to measure instrument stability.

A multi-point calibration curve was established for the analysis of Aroclors 1242 and 1254. The standards used spanned the expected range of contaminant concentrations in the samples. The analyzed samples were bracketed by the standards, with solvent rinses between standards and samples. Where possible, less concentrated standards and samples were analyzed before more concentrated standards or samples to limit the possibility of carryover (see above). Instrument response and chromatography were assessed for each standard run and compared to established norms. Remedial action (e.g., column replacement, septum and/or liner replacement, reanalysis) was taken to correct problems if

warranted. Routine maintenance and service was conducted according to the manufacturer's instructions and good laboratory practice. Log book records were used to document maintenance for each instrument.

The GC-MS was calibrated with perfluorotributylamine (FC-43) using system software calibration programs. During calibration, the resolution of the instrument was also checked by viewing the low (69 amu), mid (219) and high (502) mass fragments on the oscilloscope and adjusting the source tune as required. The MS source potentials were adjusted to produce a spectrum of decafluorotriphenylphosphine which met the specifications detailed by Eichelberger et al. (1975).

PAHs were quantified using an internal standard method. Each daily set of samples was bracketed by two analytical standard injections containing a range of appropriate surrogate standards. Response factors were calculated from the analytical standard injections and were used to obtain the concentration of individual PAHs in each sample.

Quality Control Checks

Consistent silicic acid column activity was assured by testing each batch of gel. A mixture of compounds was separated on a column made up of newly deactivated silica and the relative distributions of compounds in the column fractions were monitored. Method blanks were processed with a frequency of one per sample set to assess contamination from glassware, solvents, reagents, or hardware. Blanks contained less than 20% of the lowest measured sample concentration for each analyte.

To estimate precision, approximately 10% of the samples were analyzed in triplicate. Instrument precision, or variability, was addressed by multiple injections of a single standard or sample. Poor precision may be due to inadequate mixing of the sample prior to subsampling, inconsistent contamination or extraction procedures, or instrumental problems. For a set of replicate samples, poor precision was indicated by a Relative Standard Deviation (RSD) of greater than 20% if the analyte concentrations were at least 3 times higher than the detection limits.

Accuracy of the methods was also determined by the analysis of standard reference materials (SRMs). Two sediments from the National Institute of Standards and Technology (NIST) and the National Research Council Canada (NRCC) were analyzed as part of this study. The accuracy for each analyte was determined in reference to the certified value. When the accuracy fell outside ± 25% of the accepted value, remedial action was required. Remedial actions included re-evaluation of the chromatograms and/or other data and re-analysis.

Detection limits were calculated for each analyte. Actual analytical detection limits differed for each compound and for each sample due to differences in instrumental response, sample weight and extract volumes, and the levels of interfering compounds in the extracts.

Inorganic Compounds

Analytical Procedure

The analysis of samples for inorganic compounds was conducted according to ERLN SOPs. Prior to the removal of a sediment aliquot for analysis, the sediment and any standing water were physically stirred with teflon-coated or plastic spoons or spatulas to create a homogeneous mixture. Non-representative material was discarded if present.

All plasticware used in the sample analyses was acid-washed, rinsed with deionized water, and stored in sealed polyethylene bags or containers until used. Clean pipette tips were used for each sample to limit cross-contamination.

After the samples had been digested via sonicator (sediments) according to ERLN SOPs, the digestate was analyzed by flame Atomic Absorption Spectrophotometry (AAS) on a Perkin-Elmer 5000, graphite furnace AAS on a Perkin-Elmer 403, or Inductively Coupled Plasma (ICP) on an ARL 3410 instrument, depending on the sample concentrations and required detection limit.

Instrument Calibration and Maintenance

All analytical instrumentation was calibrated prior to the analysis of samples. A series of calibration standards of concentrations that bracketed the expected sample concentrations were prepared and analyzed to generate a calibration curve. Calibrations were checked periodically (after approximately 5 samples) throughout the course of the analyses by the injection of a calibration standard.

All equipment was maintained according to manufacturer's recommendations and/or good laboratory practice. Maintenance records were kept in a log book.

Quality Control Checks

Method or procedural blanks (two) were carried through all digestion and analysis steps as a measure of reagent or instrumental contamination. The concentrations of analytes in the blanks were not extensive (i.e., never greater than 20 percent of sample concentrations), so no remedial action was required.

A Standard Reference Material (SRM) from NRCC was analyzed to assess digestion efficiency and accuracy of the method. Precision was monitored on approximately 10% of the samples through replication. Separate aliquots (3) of a homogenized sample were analyzed independently. For the set of triplicate samples, the RSD for each analyte was less than 10%.

Instrument variability was addressed by the analysis of each sample in triplicate. Each digestate was analyzed three times and the mean and standard deviation were reported.

The addition of known amounts of surrogate compounds (spikes) to one sample allowed for an estimate of overall recovery. The spiked concentration added was approximately equal to the concentration found in the unspiked sample. The recovery of the spike was 80-110% of expected levels for most analytes. Recoveries outside this range were determined not to be indicative of a problem and therefore no corrective action was taken.

Detection limits were calculated for each analyte. The actual detection limits differed for each compound and for each sample due to differences in instrumental response, sample weight, and the levels of interferences. Further, detection limits varied with the different instrumentation, i.e., flame AAS versus ICP.

TOXICITY TESTS

Organism Collection and Holding

Ampelisca were collected from tidal flats in the Pettasquamscutt (Narrow) River, a small estuary flowing into Narragansett Bay, Rhode Island. Surface sediments were scooped and amphipod tubes were sieved with 0.5 mm screens and transferred to the laboratory with a small amount of sediment from the collection site. In the laboratory, amphipods were removed from their tubes by vigorous washing with a stream of water in a plastic bucket. The amphipods were poured off with the surface water onto a 0.5 mm screen, and were then further graded by size with a series of 1.5mm, 1.0mm, and 0.5mm screens. Those retained on the 1.0 mm screen were selected for use in the toxicity tests.

Rhepoxynius were obtained from the shallow subtidal zone of a southern Rhode Island coastal pond, Ninigret Pond (NIN). Surface sediments (8-10 cm) containing amphipods are scooped, by shovel, into 1.0 mm sieves. Amphipods were sieved in the field and transferred to the laboratory in a small amount of sediment from the collection site. In the laboratory, they were sieved on a 1.0 mm mesh screen with flowing sea water.

Prior to testing, amphipods were maintained in the laboratory in

pre-sieved uncontaminated sediment from the collection site and flowing filtered seawater. They were acclimated to the assay temperature at the rate of 2-4°C per day. During acclimation, Ampelisca were fed, ad libidum, the laboratory cultured diatom, Phaeodactylum tricornutum. Rhepoxynius were not fed.

Exposure Conditions

The amphipods were exposed to test sediments for 10 days using the standard method for conducting acute sediment toxicity tests with amphipods that is currently under review by ASTM (in prep). The exposure containers were one-liter beakers containing approximately 175 mL of test sediment and 725 mL of overlying seawater. The test was conducted under static conditions, with the overlying seawater gently aerated via a 2 mL glass pipette inserted above the sediment surface. Lighting was continuous to inhibit the amphipods' swimming activities. The test was conducted at 20°C and at salinities greater than 28 ppt.

Test Procedure

Each test sediment was press-sieved through a 2.0 mm screen to remove large debris and potential predators (Redmond and Scott 1989) and thoroughly homogenized before addition to the exposure chambers. The day before the test began, sediments were mixed and added to the exposure containers, and the containers were filled to the 900 mL level with filtered seawater. A glass petri dish attached to a glass rod held close to the sediment surface was used when adding the seawater to minimize disturbance of the sediment. Exposure containers were placed in a water bath, and air delivery was initiated. The addition of sediments prior to the start of the test ensured that surface sediments were oxydized.

On the following day, amphipods were sieved from holding containers through a stainless steel screen (0.5 mm for Ampelisca and 1.0 mm for Rhepoxynius) using 20°C filtered seawater and collected as described above. Amphipods were distributed sequentially into 100 mL plastic cups containing 20°C filtered seawater. After sorting, the cups were examined for dead, gravid or outsized animals, which were replaced with others from the same sieved population. The cups were randomized, air delivery to the exposure containers was stopped, and the amphipods added to the test chambers. After one hour, the containers were checked and any amphipods not burrowed into the sediment were replaced with others from the same sieved population. These were noted and air delivery was restarted, thus initiating the test.

Exposure containers were checked daily and the number of individuals which were dead or emerged were recorded. For Ampelisca, the condition of the amphipod tubes was also monitored. Dead amphipods that were out of their tubes and molts

were removed daily. Temperature was recorded continuously with a temperature recorder, and checked daily against a thermometer. Dissolved oxygen was measured twice during the test. Salinity of overlying seawater was measured at the initiation of each experiment and in each chamber at the conclusion of the experiment (Table 2).

After 10 days, the assay was terminated and the contents of each exposure container were sieved through a 0.5 mm screen using flowing 20°C seawater. Rhepoxynius chambers were pre-screened with a 1.0 mm sieve. Many of the amphipods were retained on this larger sieve, while most of the sediment passed through. This step expedited the counting process. Material retained on the sieves was examined under a dissecting microscope. Any chambers for which 90% of the amphipods could not be accounted for (alive, dead, or dead during the test) were recounted by another investigator as a quality assurance check. The recovered animals were counted; any missing individuals were assumed to have died and decomposed during the test and were counted as mortalities.

Experimental Design and Analysis

Each sediment was tested with five replicates and 20 amphipods per replicate. Amphipod acute tests were conducted on sediments collected from a total of 12 sites. Ten sediments, representing a wide range of physical and chemical properties, were collected from NY/NJ Harbor sites designated by EPA Region II and two sediments came from the Region II Reference and Control sites. The 12 sediments were collected on two different occasions and were thus tested in 2 sets of 6 sediments each. A performance control (Long Island Sound (LIS) reference sediment for A. abdita and Ninigret Pond sediment for R. hudsoni) was run for each set.

Based on the acute test results, the two most toxic sediments, those from Stations 8 and 10, were selected for use in LC50 tests. In these tests, a dilution series of each sediment, including four treatments and a control, was achieved by mixing the toxic sediment with a suitable diluent sediment on a volume:volume basis (see below). The diluent sediment was made by mixing sediment from LIS with sediment from Ninigret Pond, taking into consideration the water content, wet density, and % sand content of the LIS and NIN sediments. The goal was to approximate the sand to fine ratio of the two test sediments in the diluent sediments. Two replicates of each treatment were run in the LC50 tests. Performance controls were also included for each species as described above.

Table 2. Amphipod toxicity test parameters.

PARAMETER	SAMPLING FREQUENCY	TEST REPLICATE SAMPLED	IMMEDIATE PROCESSING OR MEASUREMENT
Survival	Daily	all chambers	record # of dead amphipods and remove
Emergence	Daily	all chambers	record # amphipods on sediment or water surface
Condition of test organisms	Daily	all chambers	record molting, amphipods moribund, condition of tubes
Temperature	Daily	water bath	record thermometer measurement to nearest 0.5°C
	Continuous	water bath	observe temperature recorder chart for variation
Salinity	twice/ test	seawater added, and all chambers at conclusion of test	record refractometer measurement to nearest 0.5 parts per thousand
Dissolved oxygen	twice/ test	all chambers	record meter reading to nearest 0.1 ppm

DILUTION SERIES FOR LC50 TREATMENTS

Treatment	Test Sediment (ml)	Diluent Sediment (ml)
Control	0	1000
12.5%	125	875
25.0%	250	750
50.0%	500	500
100.0%	1000	0

Statistical differences in toxicity were determined using one way analysis of variance (ANOVA) on proportional mortalities which were transformed using the arcsine square root transformation. Treatment differences were assessed using the appropriate multiple range tests. LC50s were calculated using the trimmed Spearman-Karber method. Spearman's rank correlations were determined between toxicity and bulk sediment chemistry, in which organics data were normalized to the total organic carbon content, and metals data were normalized to proportion of fines.

RESULTS

SEDIMENT PHYSICAL PARAMETERS

Based on the grain size analyses, the sediments collected from the twelve sites in the NY/NJ area can be classified into four types: sand, silty sand, sandy silt and mud (Table 3, Figure 1). High sand content (85-100%) characterizes Stations 1 (Kearney Point), 5 (Tremley Point), 7 (Ambrose Channel), and 9 (Red Hook Channel). High clay content (14-33%) characterizes Stations 2 (Elizabeth Channel) and 6 (Ward Point). The remainder of the sites (Station 3 Goethals Bridge, 4 Gulfport, 8 Gowanus Canal, and 10 Newtown Creek) are characterized by high percentages of silt (39-70%). The Reference site was 100% sand, and the Control site was 55% sand.

These sediments ranged from very poorly sorted sands to very poorly sorted and extremely poorly sorted silty sands, sandy silts and muds. Water contents, on a wet weight basis, ranged from 12% for the sandy station in Ambrose Channel to 71% for muds from Newtown Creek.

The dilution experiments were conducted with Two of the most toxic sediments (Station 8 and 10) using a combination of Long Island Sound Reference and Ninigret Pond sediments as the diluent. These two performance control sediments were mixed in proportions that approximated the sand/silt/clay fractions of the

Table 1. Station locations for EPA Region II sediment collections.

LOCATION	STATION	LATITUDE	LONGITUDE
Kearny Point	1	40°42'34"N	74°06'59"W
Elizabeth Channel	2	40°40'30"N	74°08'09"W
Goethel's Bridge	3	40°38'06"N	74°11'50"W
Gulfport	4	40°37'26"N	74°12'17"W
Tremley Point	5	40°35'30"N	74°12'14"W
Ward Point	6	40°29'48"N	74°15'13"W
Ambrose Channel	7	40°30'32"N	73°57'11"W
Gowanus Canal	8	40°40'06"N	73°59'59"W
Red Hook Channel	9	40°39'54"N	74°01'24"W
Newtown Creek	10	40°44'16"N	73°56'53"W
Milton Harbor (WLIS)	Region II Control	40°56'08"N	73°42'08"W
Reference	Region II Reference	40°20'13"N	73°52'11"W

Table 3. Physical properties of sediments collected in the New York/New Jersey Harbor area for sediment toxicity testing.

STATION	LOCATION	SEDIMENT TYPE	%SAND	%SILT	%CLAY	MEDIAN (ϕ)	MEAN (ϕ)	SORTING	SKEWNESS	KURTOSIS	% WATER
1	Kearny Point	sand	90.0	8.1	1.8	1.50	2.10	2.64	1.74	3.73	17.5
		"	92.0	6.4	1.6	1.50	2.10	2.67	1.73	3.67	18.3
2	Elizabeth Chan.	sandy silt	21.3	64.0	14.7	6.95	6.20	6.54	1.13	1.34	42.7
		"	14.9	59.7	25.4	6.35	6.10	6.35	1.12	1.32	45.0
3	Goethals Bridge	silty sand	60.5	39.5	-	2.90	3.40	3.79	1.28	1.73	45.0
		"	57.1	41.5	1.4	3.15	3.50	3.90	1.26	1.67	44.4
4	Gulfport	sandy silt	31.1	65.5	3.4	5.49	4.80	5.20	1.18	1.43	62.4
		"	30.1	67.7	2.2	5.45	4.60	4.92	1.16	1.39	62.1
5	Tremley Point	silty sand	85.2	13.0	1.8	1.40	2.20	2.81	1.83	3.69	33.1
		"	67.9	32.1	-	1.85	2.80	3.30	1.47	2.23	29.9
6	Ward Point	mud	9.2	57.3	33.5	7.50	6.6	6.84	1.09	1.24	63.8
		"	9.0	60.1	30.9	6.80	6.5	6.76	1.10	1.26	64.1
7	Ambrose Chan.	sand	100.0	-	-	1.50	1.80	1.80	1.31	1.67	13.6
		"	100.0	-	-	1.65	1.80	1.79	1.31	1.67	12.8
8	Gowanus Canal	sandy silt	47.0	51.2	1.8	4.25	4.30	4.64	1.19	1.48	59.1
		"	31.5	67.0	1.5	4.90	4.50	4.71	1.12	1.32	58.7
9	Red Hook Chan.	sand	100.0	-	-	1.90	2.10	2.20	1.15	1.37	20.6
		"	98.7	1.3		1.90	2.10	1.96	nd	nd	19.7
10	Newtown Creek	sandy silt	27.2	72.3	0.5	5.75	5.30	5.53	1.08	1.20	71.3
		"	13.2	83.9	2.9	5.70	5.20	5.33	1.08	1.20	68.5
REFERENCE		sand	100.0	-	-	1.70	2.00	1.96	1.24	1.49	16.1
CONTROL		muddy sand	54.1	29.1	16.8	3.65	4.20	4.57	1.22	1.60	52.1
		"	55.1	44.9	-	3.65	3.90	4.03	0.99	0.72	53.2

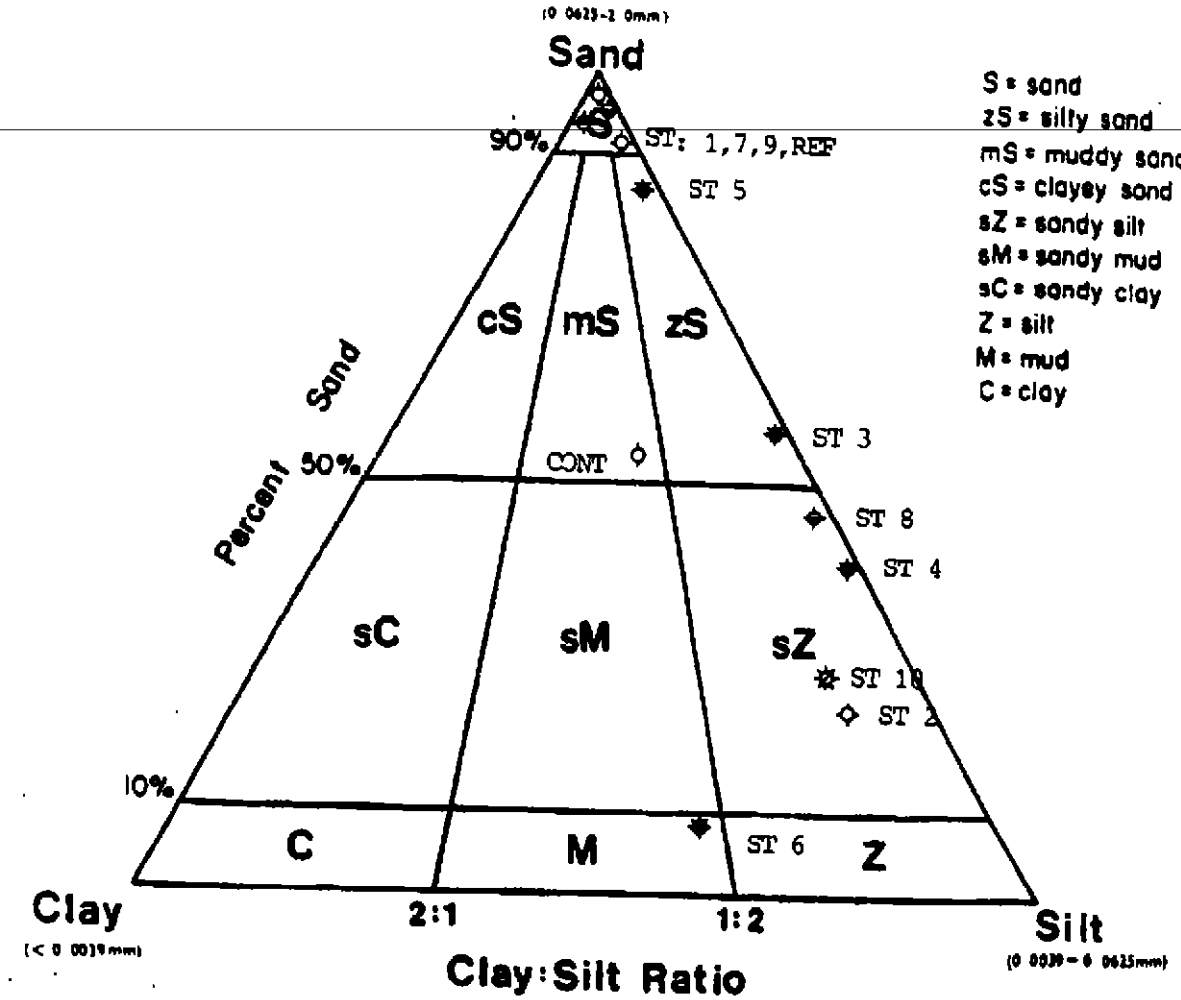

Figure 1. Grain size distribution for sediments collected for Region II sediment toxicity testing.

two test sediments. The size distributions for the four dilutions of each sediment are shown in Table 4. As the table indicates, there is very good agreement between the diluted sediments and the their respective test sediments.

SEDIMENT CHEMICAL PARAMETERS

As with grain size, there was a wide range in contaminant concentrations in these sediments (Table 5). The compounds selected for analysis are listed in Appendix Table 1. Total PCBs (Appendix Table 2) were either not detected or found at low concentrations at the sandy sites. PCB contamination was most pronounced at Stations 3, 4, 8, and 10 (Goethals Bridge, Gulfport, Gowanus Canal and Newtown Creek, respectively), exhibiting concentrations in the parts per billion range. Concentrations of the pesticides, chlordane, DDD and DDT (Appendix Table 3) were also higher at these same stations, and highest at Station 5 (Tremely Point). Dioxin levels were highest at Station 3 and furans at Stations 3 and 10 (Appendix Table 4). PAHs were most abundant at Stations 8, 10 and 5 (Appendix Table 5).

The sites identified as the most contaminated with organic compounds (Stations 3, 4, 8, and 10) also exhibited the highest overall metal contamination (Table 5). High concentrations of zinc and lead were also found at Station 6 (Ward Point). Metal concentrations were the lowest at the stations with a very high sand fraction (Stations 1, 7, and 9). The mean total organic carbon content ranged from 0.12 to 4.74%, and was lowest at the sand stations (1, 7, and 9) and progressively higher at the sandy silt stations (8 and 10).

Total organic carbon (Appendix Table 4) was generally low, varying from <1.0 % at the sandy sites to between 3 and 5 % at the mud and muddy sand sites.

SEDIMENT TOXICITY

The toxicity of the 12 sediments to Ampelisca and Rhepoxynius is summarized in Table 6, and provided in detail in Appendix Table 6. The two test series with each species met the acceptance criteria of performance control mortality less than 10%. Both species also experienced no significant toxicity in sediments from the Reference and Control sites.

Analysis of variance ($p < 0.05$) was conducted to identify those sediments with toxicities for each species significantly different from that of the regional Reference site. The Ampelisca test series identified eight of ten stations as toxic, while significant Rhepoxynius toxicity was found at only five of the ten stations.

Table 4. Percent sand/silt/clay for the sediment dilutions of Station 8 (Gowanus Channel) and Station 10 (Newtown Creek) sediments. Dilution sediments were comprised of combinations of the two performance control sediments from Long Island Sound and Ninigret Pond.

Concentration (%)	% Sand	% Silt	% Clay
Station 8			
12.5	59.7	30.8	9.5
25.0	57.2	37.8	5.0
50.0	54.3	40.6	5.1
100.0	50.5	49.5	0.0
Control	53.8	35.9	10.3
Station 10			
12.5	29.6	55.6	14.8
25.0	25.9	61.1	13.0
50.0	24.9	62.1	13.0
100.0	27.2	72.3	0.5
Control	29.0	52.6	18.4

Table 5. Concentrations of selected chemicals in sediments collected in the New York/New Jersey Harbor area for sediment toxicity testing. Units are expressed as dry weight. nd = not detected.

STATION	PCBs (ppb)	CHLORDANES (ppt)	DDD (ppt)	DDT (ppt)	PAHs (ppm)	DIOXIN (ppt)	Cu (ppm)	Cd (ppm)	Pb (ppm)	Zn (ppm)	Cr (ppm)	Ni (ppm)
1	75.1	2.13	3.24	0.52	3.83	57.5	23.1	0.431	21.6	52.8	25.5	10.6
2	462.	14.1	24.9	0.71	12.7	74.9	85.8	1.66	67.2	150.	73.0	25.2
3	2960	23.2	122.	32.3	37.2	131.	509.	8.61	250.	464.	187.	48.7
4	2450	9.63	149.	116.	30.5	nd	527.	8.73	278.	542.	233.	54.9
5	214.	45.8	1340	1130	86.4	nd	120.	0.880	120.	180.	27.7	13.7
6	376.	11.3	37.2	3.61	12.2	31.0	255.	1.98	189.	390.	111.	40.2
7	nd	nd	nd	nd	0.096	nd	0.90	0.023	5.6	10.4	5.3	2.42
8	8770	27.8	4.37	2.70	304.	nd	303.	13.2	370.	557.	147.	59.1
9	19.6	0.09	1.07	0.84	6.22	nd	18.9	0.191	32.6	49.8	18.4	28.9
10	6560	31.9	7.74	17.5	152.	nd	666.	14.9	468.	986.	260.	109.
CTL	167.	3.39	2.92	0.63	5.44	4.02	85.8	1.42	52.2	181.	60.0	24.1
REF	nd	0.09	0.14	nd	0.144	nd	11.0	0.034	8.1	18.0	9.0	3.15

concentrations given on a dry weight basis

Table 6. Mortality of the amphipods Ampelisca abdita and Rhepoxynius hudsoni exposed to sediments from the New York/New Jersey Harbor area. Standard deviations are in parentheses. Asterisks indicate significant differences as determined by ANOVA between test sediment and the Reference site sediment at $p<0.05$. Performance controls used for A. abdita came from Long Island Sound, those for R. hudsoni were from Ninigret Pond, RI.

Station	Mortality (%)			
	A. abdita		R. hudsoni	
1	35*	(17.7)	9	(4.2)
2	80*	(17.7)	9	(6.5)
3	100*	(0)	41*	(10.2)
4	100*	(0)	19	(8.0)
5	100*	(0)	27*	(8.4)
6	31*	(4.2)	9	(6.5)
7	11	(6.5)	12	(7.6)
8	100*	(0)	100*	(0)
9	12	(9.7)	11	(5.5)
10	100*	(0)	77*	(17.2)
Reference	7	(6.7)	11	(9.6)
Control	8	(5.7)	6	(5.5)
Per. Cont. 1	7	(4.5)	7	(4.5)
Per. Cont. 2	1	(2.2)	3	(4.5)

Analysis of variance using Duncan's procedure was used to rank the toxicity of all stations for each species. The station ranks and the breakdown of statistical differences are shown below. Stations not significantly different from each other are underlined by the same line.

Ampelisca:

8 10 3 5 4 2 1 6 9 7 Cont Ref LIS

Rhepoxynius:

8 10 3 5 4 7 9 1 Ref 2 6 Cont Nin

Both species rank the five most toxic sediments equally. The Ampelisca test distinguished Stations 7 and 9 as non-toxic, but the Rhepoxynius test also identified Stations 1, 2, 4 and 6 as non-toxic. The Ampelisca response classied six stations as extremely toxic (8, 10, 3, 5, 4, 2) with mortalities greater than 75%, two as moderately toxic (1, 6) with mortalities between 75% and 25%, and two as marginally toxic or non-toxic (9, 7) with mortalities less than 25% (Long 1985). The Rhepoxynius response identified two stations (8, 10) as extremely toxic, two (3, 5) as moderately toxic, and six (4, 7, 9, 1, 2, 6) as non-toxic by this classification.

The significance of the difference in toxicity between each species across all toxic stations was tested using the Wilcoxon signed-ranks test. Using the data from the seven cases where there was significant toxicity in either species, this non-parametric test showed Ampelisca to be more sensitive than Rhepoxynius ($p<0.05$). The one case where both species experienced 100% mortality is described below.

Dilution experiments to determine LC50s can be used to compare the relative sensitivity of highly toxic sediments both within and among species. Since there were five sediments causing 100% mortality in Ampelisca, the two sediments most toxic to Rhepoxynius, those from Stations 8 and 10, were chosen for these tests. The mean percent sand/silt/clay for the five treatments of each sediment are (from Table 4):

Station 8: 55.1/38.9/6.0
Station 10: 27.3/60.7/11.9

The mortality data are shown in Appendix Table 7 and the LC50s in Table 7. Ampelisca was significantly more sensitive to both sediments, and both species identified Station 8 sediments to be more toxic than those from Station 10.

The data in Table 6 and Appendix Tables 6 and 7 indicate that there was little response by either amphipod species to sediment grain size variations. No significant Ampelisca mortalities were observed in very sandy sediments from Stations 7, 9, and the Reference site. Similarly, Rhepoxynius exhibited no toxic response to high silt-clay sediments from Stations 2 and 6.

These observations are corroborated by Spearman's rank correlation analysis comparing amphipod mortality with sediment physical and chemical parameters (Table 8). Ampelisca toxicity was significantly correlated ($p<0.05$) with 11 of 13 chemical contaminants measured. It was also highly correlated with iron, silt content and organic carbon. The percent silt and TOC are also highly correlated with the contaminant suite. The lack of sensitivity in Rhepoxynius is again seen here, as mortality was correlated only with total PCBs and PAHs. Less significant positive correlations were found for lead and cadmium.

DISCUSSION

The purpose of this study was to demonstrate the utility of the amphipod ten-day sediment toxicity test for evaluating the potential hazards associated with contaminated sediments. A primary issue related to the application of these tests is the response of the test organism to sedimentary variables, such as grain size and organic carbon content.

The sediments for these tests were selected to span a broad range of grain sizes, from sandy sediments to mud. The two "non-contaminated" sediments, Reference and Control, also exhibit divergent grain sizes, which caused no significant mortality in either amphipod species. The insensitivity to grain size differences is also demonstrated in Ampelisca's lack of response to sediments from Stations 7 and 9, each with a sand fraction greater than 90%. In the San Francisco Bay bioassay intercomparison, Long and Buchman (1989) also found Ampelisca to be insensitive to grain size variations. Rhepoxynius was insensitive to fine sediments from Stations 2 and 6. It, thus, appears that either species may be utilized over a wide range of grain sizes without concern for sedimentologic effects or the need to apply normalizing factors such as those described by DeWitt et al. (1989) for R. abronius.

The insensitivity of Ampelisca to variations in grain size may be related to the its tube-building habit. It does not burrow into

Table 7. LC50s resulting from exposures of Ampelisca abdita and Rhepoxynius hudsoni to dilutions of sediments from Stations 8 and 10. Values are calculated using the trimmed Spearman-Karber method. 95% confidence limits are in parentheses.

	LC50 %	
	Station 8	Station 10
A. abdita	< 12.5	15.7 (14.0-17.6)
R. hudsoni	33.2 (28.5-38.7)	75.5 (67.0-85.2)

Table 8. Spearman rank correlation coefficients between Ampelisca abdita and Rhepoxynius hudsoni mortalities and sediment physical and chemical parameters. Organic and metal concentrations were normalized to TOC content and percent fines, respectively. An asterisk denotes statistically significant coefficients at $p<0.05$. A double asterisk denotes significant coefficients at $p<0.10$.

Parameter	Ampelisca	Rhepoxynius
Total PCBs	0.90*	0.62*
Total PAHs	0.82*	0.69*
Chlordane	0.81*	0.41
DDD	0.62*	0.06
DDT	0.78*	0.45
Dioxin	0.02	-0.50**
Furan	0.20	-0.14
Copper	0.70*	0.34
Zinc	0.70*	0.36
Chromium	0.70*	0.36
Lead	0.79*	0.50**
Nickel	0.68*	0.31
Cadmium	0.77*	0.50**
Manganese	0.42	-0.08
Iron	0.60*	0.21
Sand	-0.47	0.01
Silt	0.70*	0.31
Clay	0.20	-0.42
TOC	0.69*	0.17

sediments, except when necessary to build tubes. The tube, open only at the top end when feeding, extends 1-4 cm into the surface sediments. Observations of dye injected into subsurface sediments reveals that, when not feeding, the amphipod respires pore waters that are transported into the base of the tube by Ampelisca's respiratory currents (D. Robson et al., in prep.). This organism is thus a good pore water sampler, and its response to contaminants is not influenced by the vagaries of grain size effects. The reason for R. hudsoni's tolerance of grain size variation is unknown and would be somewhat more speculative.

Should it be necessary to establish grain size controls, however, the methods employed here for the dilution experiments are applicable. The combination of natural sediments of very divergent size composition was very effective in creating dilution sediments where a constant grain size could be maintained over the entire dilution series. The use of natural sediments that the test organisms are known to tolerate obviates the need to age the sediments or add organic carbon.

There is concern that the total organic carbon concentrations of the diluent sediments would cause large variations in the TOC levels in the final sediment mixtures, which would in turn affect the bioavailability of the chemical constituents. This is probably not the case here where the Long Island Sound sediment, at about 2% TOC, is mixed with Ninigret Pond sediment, at <1% TOC. The TOC content of the desired diluent mixture, will vary between these two values depending on the proportion of each sediment that is used. Where one of the diluent sediments has a much higher TOC content, the potential for organic carbon interference could be considerable. The TOC content was not measured in the LC50 test sediments.

Another factor that could affect the toxic response to these sediments is the presence of acid volatile sulfides (AVS). It has been demonstrated that metal ions bind very strongly to AVS and, when molar AVS concentrations are less than molar metal concentrations, metal cations are available in the interstitial waters and are toxic to these two species of amphipods (DiToro et al. in press). This constituent also was not measured in these sediments.

The contaminant concentrations in these sediments are typical of estuarine areas in the northeast and elsewhere (NOAA 1988). Sediments were selected to represent a wide range of contamination, against which amphipod responses could be compared. In an attempt to evaluate the potential for biological effects as a result of exposure to the sediment contaminants found in this survey, we have applied the approach and information gathered by Long and Morgan (1990). These authors reviewed the existing literature where biological and chemical data on sediments were available in order to assess contaminant

levels at the NOAA National Status and Trends sites. For each analyte on the NOAA list, Long and Morgan calculated Effects Range - Low (ER-L) and Effects Range - Median (ER-M) values that were based on apparent effects threshold data, co-occurance analyses, equilibrium partitioning, background approaches and spiked sediment bioassays, when available.

The ER-L and ER-M values are shown in Table 9 for the 12 contaminants in Table 5. Using the median effects range (ER-M) concentrations, seven stations are identified as having the potential for biological effects. In descending order of rank importance, the stations (and the number of contaminant concentrations exceeding the ER-M value) are: 10(8), 8(7), 4(6), 3(6), 6(2), 5(2), and 2(1). Stations 1, 7, 9, and the Reference and Control sites had no concentrations exceeding the ER-M.

The pattern of Ampelisca toxicity follows these predictions quite well in that significant toxicity was found at all of these stations; 100% mortality was found in the four top-ranked stations. Toxicity was observed at Station 1, where concentrations are not high enough to predict biological effects. This station is one of the three stations with the highest sand fractions, and has, with the exception of DDT, lead, and nickel, the highest contaminant concentrations of the other sand sites, Stations 7 and 9. It is possible that the contaminants in this sandy sediment are more bioavailable than are those of equal concentrations in silty sand or mud because of the lower TOC concentrations, and decreased contaminant binding capacity.

The toxicity pattern for Rhepoxynius is similar, but only matches four of the seven sediments (Stations 10, 8, 3, 5) that are predicted to elicit biological effects. The reduced sensitivity, as compared to Ampelisca, is consistent with previous studies on metal toxicity using water only exposures. In those 96-hour tests with both species, Ampelisca was consistently more sensitive (W. Berry, SAIC-Narragansett, pers. comm.). Direct comparisons between Ampelisca and the more commonly used R. abronius are more rare. The bioassay comparison study using San Francisco Bay sediments found Ampelisca to be less sensitive than R. abronius (Long and Buchman 1989), but these differences may have been due to the widespread response of the latter species to grain size (Dewitt et al. 1988). In a comparison of the two species to 51 sediment treatments from Oakland Harbor, Word et al. (1989) found Ampelisca to be slightly more sensitive than R. abronius when static exposures were used. Overall, the available data indicates that Ampelisca is at least as sensitive as R. abronius. This study demonstrates that it is more sensitive than R. hudsoni.

The ANOVA and mutiple range test for the responses of both species detected differences between the performance control and the Reference sediment. These differences highlight the fact that, using five replicates per treatment, very small differences

Table 9. Effects Range - Low (ER-L) and Effects Range - Median (ER-M) concentrations for the contaminants measured in Stations 1-10 and the Control and Reference sites. Values are after Long and Morgan (1990).

Compound	ER-L	ER-M
Total PCBs (ppb)	50.0	400.0
Chlordane (ppb)	0.5	6.0
DDD (ppb)	2.0	20.0
DDT (ppb)	1.0	7.0
Total PAHs (ppb)	4.0	32.0
Copper (ppm)	70.0	390.0
Zinc (ppm)	120.0	270.0
Chromium (ppm)	80.0	145.0
Lead (ppm)	35.0	110.0
Nickel (ppm)	30.0	50.0
Cadmium (ppm)	5.0	9.0

between treatments (<10%) can be detected. This was also the case for Rhepoxynius mortality at Stations 4 (19% mortality) and 5 (27% mortality). As would be the case in a permit application, the test sediments were statistically compared with the Reference site sediments. While small differences between a test sediment and a performance control may be detected, it appears that only mortalities greater than 20% should be considered significant, because a maximum mortality of 10% is allowable for control responses and an additional 10% mortality approximates the range of statistical detection.

Ideally, mortality in Reference site sediments should not exceed 10%, but, as was found with Rhepoxynius, that condition may not always hold. In this instance, any mortality greater than 20% which is statistically different from the Reference site should be considered significant.

The term "significant" can be related to statistical or biological importance. The former is quantitative and clearcut, while the latter is speculative, ambiguous, and undefined. This is an unfortunate circumstance, as regulatory principles should rely on biological significance more strongly than is now the case. It is clear that 100% mortality is biologically important. The same can be said for a 50% mortality. But, what is the biological significance of mortalities between 20 and 50%?

One way to address this question is through the use of demographic models that relate basic life history characteristics (birth and death rates, fecundity, survival) to the potential for population growth, through the calculation of the intrinsic rate of population growth, r. This approach has been applied to chronic test data on Ampelisca exposed to contaminated sediments in suspension (Scott and Redmond 1989). The ten-day exposure period for this acute test represents approximately one third of Ampelisca's life cycle. Mortality for two successive ten-day periods could be assumed to be equal and then used as input to a model. The relationship between exposure duration and toxicity would have to be determined to verify this assumption. Other necessary information would include the responses in the fecundity of this amphipod to changes in population density, since many species compensate for lower population density (acute mortality, in this case) by increasing fecundity. Work is progressing in the collection of some of these data. There is also a critical need for more direct measures of sublethal and chronic effects in laboratory tests that can be used to predict chronic effects in natural communities (Scott 1989).

The emergence data for both species were examined for use as an additional assessment endpoint. Several problems with the use of emergence became immediately apparent. First, the variability among treatments was extremely large and, second, there is no simple way to quantify this parameter. Using the total number of

animals emerged as a metric is misleading because low emergence may result from high mortality, as is the case in these tests. There have also been cases where the sediment is so toxic that the amphipods immediately die in the sediments and emergence is low. Development of a measure using proportion of amphipods emerged requires that the number of survivors be determined on a daily basis, but exact estimates of survivorship are only available at the end of the test. In a previous study, the emergence response for Ampelisca and the emergence and reburial response in R. abronius were found to be relatively insensitive, and to provide no more information than survival alone (see also Long and Buchman 1989).

CONCLUSIONS AND RECOMMENDATIONS

1. Both Ampelisca and Rhepoxynius exhibited excellent control survival, and responses of each species indicated that the Region II Reference and Control sites are suitable for these purposes.

2. Both Ampelisca and Rhepoxynius identified the most toxic sediments, however, the former species was significantly more sensitive at all sites with toxic sediments. Ampelisca is thus recommended as the preferred species.

3. Sediment particle size did not affect the sensitivity of these amphipods, and either species can be used over the range of sediment particle sizes described here. The use of a grain size control, prepared as in this study, is recommended.

4. The LC50 test is a simple way to determine the relative toxicity of two highly contaminated sediments, provided that care is taken to mitigate potential grain size effects.

5. Ampelisca toxicity is more closely correlated with contaminant concentrations than is Rhepoxynius. Observed toxicity in this species is also more concordant with what would be predicted based on contaminant concentrations.

6. The level of detectable differences for these amphipod tests are quite low (<10% mortality). It is recommended that only mortalities greater than 20% that are statistically different than those in the Region II Reference site be considered biologically significant.

7. The use of amphipod emergence as a sublethal endpoint is discouraged because of difficulties in its measurement and interpretation.

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Appendix Table 1. Organic compounds selected for analysis in sediments collected for Region II sediment toxicity testing.

PESTICIDES

Hexachlorobenzene (HCB)
a-hexachlorocyclohexane (a-BHC)
a-hexachlorocyclohexane (a-BHC)
g-hexachlorocyclohexane (g-BHC)
a-chlordane (a-CHLO)
g-chlordane (g-CHLO)
p,p'-DDE
p,p'-DDD
p,p'-DDT

PCBs

2,2',5,5'-PCB (CB052)
2,2',4,4'-PCB (CB047)
2,2',4,5,5'-PCB(CB101)
2,2',3,5,5',6-PCB(CB151)
2,3',4,4',5-PCB (CB118)
2,2',4,4',5,5'-PCB (CB153)
2,2',3,4,4',5'-PCB (CB138)
2,2',3,3',4,4'-PCB (CB128)
2,2',3,4,4',5,5'-PCB (CB180)
2,2',3,3',4,4',5,6-PCB (CB195)
2,2',3,3',4,4',5,5'-PCB (CB194)
2,2',3,3',4,4',5,5'6-PCB (CB206)
CL10-PCB (CB209)
Aroclor 1242
Aroclor 1254

Polycyclic Aromatic Hydrocarbons (PAHs)

Fluorene (FLU)
Phenanthrene (PHEN)
Anthracene (ANT)
C1 homologs of phenanthrene and anthracene (C1P+A)
C2 homologs of phenanthrene and anthracene (C2P+A)
C3 homologs of phenanthrene and anthracene (C3P+A)
C4 homologs of phenanthrene and anthracene (C4P+A)
Fluoranthene (FLUO)
Pyrene (PYR)
Benz[a]anthracene (B[a]A)
Chrysene (CHRY)

Sum of benzofluoranthenes (BFLU)
Benzo[e]pyrene (B[e]P)
Benzo[a]pyrene (B[a]P)
Perylene (PERY)
Sum of molecular weight 276 PAHs (MW276)
Sum of molecular weight 278 PAHs (MW278)
Coronene (CORO)
Sum of molecular weight 302 PAHs (MW302)
10-Benzotriazole (10-BT)
Chloro-Benzotriazole (Cl-BT)

Appendix Table 2. PCB congener concentrations (ng/g dry weight) in sediments collected for Region II sediment toxicity testing. nd = not detected.

STATION	Cb052	Cb047	Cb101	Cb151	Cb118	Cb153	Cb138	Cb128	Cb180	Cb195	Cb194	Cb206	Cb209
1	2.15	1.31	2.57	0.670	3.27	3.13	2.27	0.322	1.47	0.187	0.435	0.395	0.66
2	9.59	7.14	12.9	3.06	18.8	14.8	13.8	0.729	7.76	0.932	2.08	2.17	3.66
3	107.	60.5	79.0	13.8	102.	64.3	65.1	3.56	32.6	3.61	8.93	7.42	12.6
4	70.5	40.8	69.6	17.4	95.2	77.5	68.8	4.11	38.5	4.10	10.3	7.92	10.9
5	5.63	3.22	6.24	1.63	8.94	8.26	6.90	0.756	4.57	0.665	1.71	1.94	2.03
6	5.82	6.80	9.80	2.55	15.8	14.7	11.4	1.08	7.49	ND	2.64	6.14	8.49
7	ND	0.079	0.084	ND	0.073	0.088	0.049	ND	ND	ND	ND	ND	0.027
8	159.	128.	172.	5.09	171.	221.	219.	55.0	138.	15.9	ND	16.6	12.0
9	0.567	0.482	0.839	0.219	0.914	1.18	0.880	0.125	0.594	0.102	0.201	0.223	0.373
10	93.9	80.2	137.	9.38	138.	179.	160.	41.9	103.	13.2	0.609	18.0	19.7
CTL	2.21	1.13	5.58	1.36	7.27	7.74	7.76	0.558	3.07	0.481	1.25	1.01	1.78
REF	0.11	0.102	0.233	0.062	0.276	0.347	0.251	0.117	0.160	0.031	0.053	0.064	0.106
BLANK	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.132

Appendix Table 3. Pesticide concentrations (pg/g dry weight) in sediments collected for Region II sediment toxicity testing. nd = not detected.

STATION	A-BHC	G-BHC	A-CHLOR	G-CHLOR	P,P'DDE	P,P'DDD	P,P'DDT	HCB
1	nd	nd	1.48	0.650	1.61	3.23	0.518	0.134
2	0.470	0.537	10.7	3.37	3.91	24.9	0.710	0.493
3	1.60	nd	16.3	6.92	180.	122.	32.3	14.9
4	0.546	1.10	5.75	3.87	136.	149.	116.	5.49
5	2.67	nd	28.7	17.1	2.28	1340	1130	1.24
6	0.455	0.612	7.79	3.35	11.9	37.2	3.61	0.209
7	nd	nd	nd	nd	nd	nd	nd	nd
8	nd	nd	20.0	7.78	40.1	2.63	2.70	nd
9	nd	nd	0.494	nd	0.195	1.06	0.841	0.027
10	nd	nd	11.9	20.0	48.3	nd	17.5	12.0
CTL	nd	nd	2.42	0.968	nd	2.92	0.625	0.070
REF	nd	nd	0.093	nd	0.051	0.138	nd	0.008
BLANK	nd	nd	nd	nd	nd	nd	nd	nd

Appendix Table 4. Dioxin, furan and total organic carcon concentrations in sediments collected for Region II sediment toxicity testing. nd = not detected.

STATION	DIOXIN (pg/p)	FURAN (pg/g)	TOC (%)
1	57.5	19.4	0.45
2	74.9	50.7	1.67
3	131.	157.	2.51
4	nd	26.6	3.54
5	nd	8.68	1.42
6	31.0	68.9	3.28
7	nd	2.83	0.12
8	nd	nd	4.15
9	nd	nd	0.25
10	nd	98.9	4.74
REF	nd	0.41	0.18
CONTROL	4.02	12.9	2.50

Appendix Table 5. Concentrations of individual PAHs (ug/g dry) in sediments collected for Region II sediment toxicity testing. nd = not detected.

STATION	FLU	PHEN	ANTH	C1P+A	C2P+A	C3P+A	C4P+A	FLUO	PYR	B(a)A	CHRY	BFLU	B(e)P	B(a)P	PERY	MW276	MW278	CORO	MW302	10-BT	C1-B'
1	0.021	0.14	0.102	0.116	0.134	0.137	0.081	0.345	0.387	0.201	0.192	0.409	0.162	0.245	0.07	0.436	0.218	0.058	0.343	0.004	0.00
2	0.073	0.312	0.331	0.46	0.853	0.897	0.516	1.07	1.12	0.582	0.619	1.15	0.529	0.646	0.255	1.3	0.648	0.145	1.08	0.003	0.00
3	0.047	0.322	1.03	2.28	6.25	5.64	2.17	2.98	3.22	1.34	1.50	2.14	1.05	1.14	0.324	2.32	1.24	0.303	1.75	0.033	ND
4	0.145	0.795	0.484	1.65	3.39	3.46	1.58	2.30	2.52	1.06	1.35	2.31	1.07	1.16	0.393	2.66	1.28	0.333	2.31	0.033	ND
5	1.14	5.32	3.90	9.55	8.11	4.42	1.92	13.3	11.1	4.03	4.02	4.31	2.00	2.64	0.592	3.98	2.24	0.403	3.02	ND	ND
6	0.053	0.249	0.165	0.348	0.509	0.567	0.376	0.796	0.941	0.495	0.586	1.31	0.615	0.678	0.348	1.66	0.817	0.195	1.45	ND	ND
7	ND	0.005	0.003	0.006	0.003	0.001	ND	0.005	0.008	0.005	0.004	0.009	0.004	0.006	0.002	0.014	0.009	ND	0.005	0.001	ND
8	9.79	32.8	15.7	39.9	35.0	17.9	5.94	26.8	30.1	11.0	11.2	13.2	5.49	8.93	1.89	13.4	8.62	1.92	12.9	0.05	ND
9	0.016	0.09	0.081	0.184	0.206	0.135	0.068	0.201	0.694	0.243	0.219	0.541	0.286	0.418	0.08	0.878	0.477	0.167	1.19	0.002	ND
10	0.854	4.12	4.69	7.7	15.1	13.4	6.34	15.4	14.8	7.08	7.39	11.3	4.89	6.07	1.61	12.2	6.85	1.24	9.67	0.019	ND
CTL	0.023	0.153	0.06	0.146	0.142	0.09	0.031	0.378	0.393	0.187	0.225	0.562	0.237	0.286	0.082	0.879	0.434	0.224	0.867	0.052	0.01
REF	ND	0.004	0.002	0.004	0.004	0.003	0.002	0.009	0.01	0.005	0.007	0.016	0.008	0.006	0.005	0.022	0.009	0.004	0.012	0.001	ND
LANK	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND	0.001	ND	ND	ND	ND	ND

Appendix Table 6a. Percent mortality of amphipods Ampelisca abdita and Rhepoxynius hudsoni exposed for 10 days under static conditions to marine sediments from the Region II area. Performance control sediments were from central Long Island Sound reference site (a) and Ninigret Pond (b), R.I. There were 5 replicates per treatment per species, with 20 amphipods per replicate.

Ampelisca abdita

sediment	% mortality replicate	mean
Performance control (a)	5	7.0
	10	
	10	
	0	
	10	
STA 1	65	35.0
	30	
	25	
	20	
	35	
STA 2	60	80.0
	95	
	65	
	80	
	100	
STA 3	100	100.0
	100	
	100	
	100	
	100	
STA 4	100	100.0
	100	
	100	
	100	
	100	
STA 5	100	100.0
	100	
	100	
	100	
	100	
STA 6	30	31.0
	35	
	35	
	25	
	30	

Rhepoxynius hudsoni

sediment	% mortality replicate	mean
Performance control (b)	5	5.0
	5	
	15	
	5	
	5	
STA 1	10	9.0
	5	
	10	
	5	
	15	
STA 2	5	9.0
	5	
	5	
	10	
	20	
STA 3	50	41.0
	30	
	45	
	50	
	30	
STA 4	20	20.0
	30	
	5	
	20	
	20	
STA 5	40	27.0
	20	
	30	
	25	
	20	
STA 6	10	9.0
	15	
	0	
	5	
	15	

Appendix Table 6b. Percent mortality of amphipods Ampelisca abdita and Rhepoxynius hudsoni exposed for 10 days under static conditions to marine sediments from the Region II area. Performance control sediments were from central Long Island Sound reference site (a) and Ninigret Pond (b), R.I. There were 5 replicates per treatment per species, with 20 amphipods per replicate.

Ampelisca abdita			Rhepoxynius hudsoni		
sediment	% mortality		sediment	% mortality	
	replicate	mean		replicate	mean
Performance control (a)	0 0 0 0 5	1.0	Performance control (b)	0 0 0 5 10	3.0
Region II Control	10 15 10 5 0	8.0	Region II Control	5 15 5 0 5	6.0
Region II Reference	0 15 10 0 10	7.0	Region II Reference	25 10 15 5 0	11.0
STA 7	20 5 10 15 5	11.0	STA 7	20 10 20 5 5	12.0
STA 8	100 100 100 100 100	100.0	STA 8	100 100 100 100 100	100.0
STA 9	20 5 10 15 10	12.0	STA 9	10 10 5 20 10	11.0
STA 10	100 100 100 100 100	100.0	STA 10	90 90 70 50 85	77.0

Appendix Table 7. Percent mortality of amphipods Ampelisca abdita and Rhepoxynius hudsoni exposed for 10 days under static conditions in an LC50 assay, using sediments from the Region II area. Performance control sediments were from central Long Island Sound reference site (a) and Ninigret Pond (b), R.I. There were 2 replicates/treatment/species, and 20 amphipods/replicate.

Station 10

Ampelisca abdita			Rhepoxynius hudsoni		
sediment	% mortality		sediment	% mortality	
	replicate	mean		replicate	mean
Performance control (a)	0 0	0.0	Performance control (b)	0 10	5.0
Control	5 10	7.5	Control	0 0	0.0
12.5	45 25	35.0	12.5	5 0	2.5
25	90 95	92.5	25	10 15	12.5
50	100 100	100.0	50	14.3 20	17.2
100	100 100	100.0	100	70 75	72.5

Station 8

Ampelisca abdita			Rhepoxynius hudsoni		
sediment	% mortality		sediment	% mortality	
	replicate	mean		replicate	mean
Performance control (a)	0 0	0.0	Performance control (b)	0 10	5.0
Control	0 5	2.5	Control	0 5	2.5
12.5	85 85	85.0	12.5	10 10	10.0
25	100 95	97.5	25	30 25	27.5
50	100 100	100.0	50	70 90	80.0
100	100 100	100.0	100	100 100	100.0