IDENTIFICATION OF ACUTE TOXICANTS IN NEW BEDFORD HARBOR SEDIMENTS

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Abstract—New Bedford Harbor (NBH) is a marine Superfund site contaminated with high concentrations of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and metals. Experiments were conducted to determine the causal toxic agent(s) in pore waters from New Bedford Harbor sediments to amphipods and mysid shrimp. Chemical manipulations to characterize toxicity revealed that pore-water toxicity was organic in nature. Fractionation and subsequent mass spectral identification of peaks in the toxic fraction indicated that PCBs, PAHs, and unknown compounds were present. Comparisons of PAH LC50s and PAH concentrations in this fraction indicated that the toxicity was not due to PAHs because the PAH concentrations were much lower than the reported PAH LC50s. One unknown peak was positively identified as bis(2-ethylhexyl) phthalate, and the other tentatively identified as pyrazole. Toxicity tests and comparison of toxicity in the blank and toxic fractions eliminated the two “unknowns” as toxic causal agents. We determined the range of PCB LC50s to fall between 10 and 110 ppb for Mysisopsis bahia and Ampelisca abdita. Concentrations of PCBs for the toxic fractions ranged from 12 to 27 ppb. This range falls within our observed PCB LC50s for M. bahia and A. abdita. Based upon these PCB concentrations, we concluded that PCBs were the acute toxic agents in NBH pore waters. Other compounds in the toxic fractions, or compounds that coeluted and were undistinguishable from PCBs had minor contributions to the measured toxicity.

Keywords—Sediments New Bedford Harbor Toxicity identification/evaluation Polychlorinated biphenyls Amphipods

INTRODUCTION

Contaminated sediments exist in many harbors near industrial and heavily populated areas. These sediments are a source of organic and inorganic pollutants to coastal and estuarine systems. In addition, many of these same sediments are often targeted for dredging to maintain shipping channels. Disposal of this dredged material is often problematic because of the toxicity of the sediments. Dredging and subsequent decontamination of these toxic sediments has been suggested as a solution to disposal problems. Decontamination techniques are usually chemical-class specific, that is, methods used to remove metals are different than methods used to remove organic compounds. Therefore, risk managers must choose appropriate decontamination method(s) for their particular circumstance. In order to choose the most effective decontamination method for a particular sediment it is necessary to determine which compounds, out of possible thousands, are causing toxicity. One technique for identifying toxic compounds in contaminated sediments is assay-directed fractionation. This technique combines chemical and biological tests to determine causes of toxicity in complex mixtures. One method of assay-directed fractionation is sediment toxicity identification and evaluation (TIE). In this method, samples are tested for toxicity and then subjected to a series of chemical manipulations designed to isolate chemical classes that may cause toxicity [1–3]. After the selective removal of specific chemical classes, samples are retested for toxicity. Any changes in toxicity imply that the chemical class removed may be responsible for the toxicity of that sample. Pore-water TIEs have successfully identified certain compounds as responsible for the majority of the toxicity in freshwater sediments [4,5]. In addition to identifying causes of toxicity for remediation purposes, sediment TIEs can be useful in determining the potentially responsible parties for Superfund cleanup activities and regulating disposal of dredge materials. Finally, sediment TIEs can be used to determine the factors important to include in ecological risk assessment models (e.g., chemicals to include in fate and transport models, chemicals that drive the toxicity of systems).

Sediments in the New Bedford Harbor, Massachusetts, USA (NBH), marine Superfund site are contaminated with extremely high concentrations (percent levels in some areas) of polychlorinated biphenyls (PCBs). However, in addition to PCBs, NBH is also heavily contaminated with metals [6], polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans, and polychlorinated dibenzo-p-dioxins [7]. A large amount of published literature exists on the effects of NBH sediments, but the majority of it has been focused on bioaccumulation and reproductive effects of PCBs in the sediments [8–12]. Although PCBs are often assumed to be the cause of acute effects resulting from exposure to NBH sediments, to accurately assess the specific cause of toxicity, all toxicants present must be considered. Relatively little work has been published on the causes of acute toxicity in NBH sediments [13,14].

The objectives of this study were twofold. The first was to develop and validate the use of TIE methodology in marine sediment pore waters. The second was to characterize and identify the toxic components in NBH sediments.

MATERIALS AND METHODS

Overview

Two series of experiments were performed. In 1992, toxicity tests and Phase I (characterization) and some Phase II (identification) TIE manipulations based on published methods

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[2,15] were performed on pore waters and toxic fractions isolated from NBH sediments. In 1994, more thorough Phase II (Confirmation) [1,3] manipulations (see section on TIE chemical manipulations) and chemical analysis liquid chromatography–mass spectrometry [LC-MS] and gas chromatography–mass spectrometry [GC-MS] analysis were conducted on the toxic fractions (Table 1).

**Sediment collection and preparation**

For the 1992 experiments, intertidal NBH sediments (top 5 cm) were collected at low tide using a polyethylene handheld scoop and placed in acrid-washed glass jars. For the 1994 experiments, NBH sediments were collected using a Ponar grab (top 15–20 cm) and placed in acid-washed 4-L polyethylene buckets. The two NBH sediments were collected using differing techniques due to sampling constraints at the restricted Superfund site. The 1992 sediments were used within 2 months, the 1994 sediments were used within 8 months (G. Ankley, personal communication, USEPA, Duluth, MN, indicated no change in stored sediments for up to 18 months). All sediments were stored at 4°C in the dark until use.

To obtain pore waters, sediments were homogenized and then centrifuged (5,000 g, 4°C) for 2 h. The supernatant pore water was decanted and recentrifuged (10,000 g, 0.5 h). The second centrifugation was performed to further remove difficult-to-centrifuge particulate carbon, thereby minimizing the potential for nonlinear dose–response curves (i.e., higher pore-water concentrations may be less toxic than lower concentrations; unpublished data, USEPA, Narragansett, RI). Particulate carbon in some of the higher concentrations may decrease bioavailability of toxic organics to the organisms by interacting with the compounds. Although centrifugation, as well as any other technique used to extract pore water, may change the toxicity of pore water due to changes in the redox potential, it is a well-accepted and documented method of obtaining pore water [4,5,16]. Double centrifugation most likely does not increase changes in redox potential more than single centrifugation, but we speculate it may remove more of the colloidal material in the pore waters.

Pore-water salinity averaged 22 parts per thousand (ppt). In order to be consistent with the salinity regime for the test organisms, sufficient 100-ppt brine was added to pore water to increase the salinity to 30 ppt. The pore waters were stored in the dark at 4°C, and used within 24 h of extraction.

Long Island Sound sediment (LIS) was used as a reference for both sets of experiments. Due to the unusually high organic carbon concentration in NBH (181 mg/g)[7], it was not possible to find a sediment with a similar organic carbon concentration. The LIS has been a standard reference sediment in our laboratory [13,14,17]. It is chemically [17] and physically [18] well characterized and is used as a nontoxic holding sediment for many of our test organisms. The LIS sediments were collected in 1992 using a Van Veen grab sampler (depth 0.5 m) and stored in plastic 208.2-L (55-gallon) drums at 4°C in the dark. In 1994, the sediments were press sieved (2-mm stainless steel sieve), homogenized, and stored at 4°C in the dark until use. Prior to use, sediments were homogenized and centrifuged as above.

**Assays**

Pore waters from NBH (100%) were tested using four toxicity tests: the acute amphipod (Ampelisca abdita) and mysid (Mysisidopsis bahia) tests, the sea urchin (Arbacia punctulata) sperm cell test, and the photoluminescent bacterial test (Microtox®, Microbics Corp., Carlsbad, CA, USA). Whole sediments from NBH were also tested using three acute assays: the amphipods A. abdita and Leptocheirus plumulosus, and the mysid test. Mysid and Arbacia assays for pore waters were conducted as described in Burgess et al. [15].

For pore waters, mysid and A. abdita tests were conducted as 48-h acute static assays in 10 ml of pore water. The 48-h-old mysids were fed newly hatched Artemia ad libitum by placing one concentrated drop of Artemia (approximately 400 organisms) into each test container per day. Ampelisca abdita were field collected, fed Phaedodactyl or Tricronatum, and acclimated to 20°C for 24 h prior to testing. On the day of the test A. abdita were sieved from the sediments, sorted, and the 0.5 to 0.74-mm juveniles (organisms that passed through a

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**Table 1. Outline of tests performed**

<table>
<thead>
<tr>
<th>Year</th>
<th>Baseline</th>
<th>Phase I—six TIE manipulations&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phase II—nine C&lt;sub&gt;18&lt;/sub&gt; column elutions&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Phase II—HPLC column elutions&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LC-MS&lt;sup&gt;d&lt;/sup&gt;</th>
<th>GC-MS, GC-ECD&lt;sup&gt;e&lt;/sup&gt;</th>
<th>PCB toxicity tests</th>
<th>Phthalate toxicity tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>Pore waters</td>
<td>A. abdita</td>
<td>A. abdita</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<sup>a</sup>TIE = toxicity identification and evaluation, EDTA = ethylenediaminetetraacetic acid; six manipulations = C<sub>18</sub>, EDTA, Na<sub>2</sub>SO<sub>3</sub>, aeration, pH 7, pH 9.

<sup>b</sup>Nine elutions = Methanol : water (v:v): 50:50, 75:25, 80:20, 85:15, 90:10, 95:5; 100% methanol; 100% acetone; 100% methylene chloride.

<sup>c</sup>HPLC = high-pressure liquid chromatograph; ten 2-ml fractions from a linear 17-min gradient of 80:20 methanol : water v/v to 100% methanol.

<sup>d</sup>LC-MS = liquid chromatography–mass spectrometry.

<sup>e</sup>GC-MS = gas chromatography–mass spectrometry, GC-ECD = gas chromatography–electron capture detection, PAHs = polycyclic aromatic hydrocarbons.

<sup>f</sup>PCB = polychlorinated biphenyls.
0.75-mm screen but were retained on a 0.5-mm screen) were used. The Microtox assay was conducted as recommended by the manufacturer, except that the dilution water was replaced with 30-ppt filtered Narragansett Bay water. This water has been an acceptable control in other experiments [14]. Male and female *A. punctulata* were stimulated with electrodes to release sperm and eggs, respectively. The gametes were collected separately, diluted, and counted. Sperm were exposed to the toxic sample for 1 h, and then mixed with eggs for 20 min to allow fertilization. Fertilization, determined by the presence of a fertilization membrane, was recorded.

Initial TIE pore-water toxicity tests were performed in triplicate at 100%, 50%, and 0% v/v pore water (five organisms/replicate, three replicates). Pore waters were diluted with a 30-ppt reconstituted seawater (brine and deionized water). Baseline (test during which the sample is not manipulated) tests were performed at the same concentration as the initial but in duplicate. All TIE manipulations, and subsequent aliquots from fractionation, were performed at 100% and 0% pore waters without duplication (five organisms/concentration) because of the limited amount of pore water and extracts available.

For whole sediment exposures, 2 g of sediment was mixed with 10 ml of 30 ppt brine and deionized water. The sediment–water mixture was allowed to settle for 24 h and the two amphipod species (*A. abdita, L. plumulosa*) and the mysids were added to separate containers (five organisms/replicate, three replicates). Mysids were fed newly hatched *Artemia ad libitum*. All whole sediment tests were terminated at 96 h. Animals were sieved for recovery and mortality was recorded. Any missing organisms were considered to be dead.

Due to the relatively high sensitivity of *M. bahia* and *A. abdita* to the toxicants in the sediment, all further tests in 1992 were conducted using these two species only. In the 1992 Phase II assay testing, solid phase extraction (SPE) with a C$_{18}$ column and high-pressure liquid chromatography (HPLC) extracts were tested at 1% and 0.5% v/v in reconstituted seawater for mysids and amphipods, respectively. This concentration translates to a nominal 5 × and 10 × the original sample concentration for the C$_{18}$ extracts and HPLC extracts, respectively, for the mysids. The amphipods were tested at 2.5 × and 5 × the original sample concentration for the C$_{18}$ extracts and HPLC extracts, respectively. The 1994 experiments were conducted using only the amphipod, *A. abdita*. In 1994, C$_{18}$ extracts were tested at 0.75% (3.75 × the original sample concentration) in reconstituted seawater.

### TIE chemical manipulations

In addition to Phase I manipulations [2,15] (Table 1), we performed Phase II manipulations to further investigate toxicity removed by the C$_{18}$ column. Five hundred milliliters of pore water was passed through a C$_{18}$ column; the C$_{18}$ column was dried and then eluted with increasingly nonpolar solvent mixtures (Table 1) to obtain nine C$_{18}$ fractions. Fractions were eluted using two 7-ml aliquots of each solvent mixture, allowing the column to dry between each solvent aliquot. The two 7-ml aliquots were combined and reduced in volume to 1 ml using a Turbo Vap® Evaporator (Zymark Corp., Hopkinton, MA, USA). The acetone and methylene chloride fractions were solvent exchanged into methanol using the Turbo Vap Evaporator. Operational controls (chemical manipulations performed on clean 30-ppt reconstituted seawater and tested for toxicity) were performed for all manipulations.

### HPLC fractionation

The C$_{18}$ fractions demonstrating toxicity were combined and subjected to further fractionation by HPLC using a Waters 600 pump, Waters 600E system controller, and a C$_{18}$ reverse-phase column (Bondapak 27324; Waters, Marlborough, MA, USA). A linear 17-min gradient of 80:20 methanol : water v/v, to 100% methanol was used ending with a 3-min 100% methanol flush (1 ml/min flow). Ten 2-ml fractions were collected and the volume was reduced (as previously discussed) to 0.5 ml.

### Extract analysis

The C$_{18}$ fractions demonstrating toxicity were analyzed by three methods: gas chromatography–electron capture detection (GC-ECD), GC-MS, and high-pressure liquid chromatography–mass spectrometry (HPLC-MS).

#### GC-MS

The toxic 90% and the 95% methanol fractions from the C$_{18}$ column were analyzed by GC-MS for PAHs [7]. Concentrations of PAHs are reported as the sum of PAHs; defined as the sum of fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[123-cd]pyrene, dibenz[ah]anthracene, benzo[ghi]perylene, and coronene. Concurrently, the extracts were examined for unknown compounds by GC-MS with mass spectrometer operation in the full scan mode. Mass spectra of major peaks were compared to spectra of known compounds and to reference spectra in the NIST/EPA/NIH (Gaithersburg, MD, USA) Mass Spectral Database for tentative identification.

#### GC-ECD

Fractions exhibiting toxicity were analyzed by GC-ECD for PCBs and phthalic acid, bis(2-ethylhexyl) ester [19]. The 18 NOAA Status and Trend PCB congeners (PCB congeners IUPAC 008, 018, 028, 044, 066, 101, 118, 153, 105, 138, 187, 128, 180, 170, 195, 206, 209) were summed and reported as total PCB concentrations. Phthalic acid was quantified by linear regression of known standards.

#### HPLC-MS analyses

The toxic 90% and 95% methanol fractions from the C$_{18}$ column were analyzed on a Hewlett-Packard (HP)1090L liquid chromatograph interfaced via a HP 59980B Particle Beam to an HP 5989A mass spectrometer (Hewlett Packard, Burlington, MA, USA).

Three methods of LC-MS analyses were performed. The first was direct injection of the sample into the MS particle beam interface without any separation by LC (analogous to direct probe analysis of solid sample). Solvent was isocratic at 100% methanol. (This method was performed in order to detect compounds that would not pass through an LC column.) The second used a linear gradient with a C$_{18}$ column (3.9 × 300 mm u-Bondapak, 10-µm particle size, 125-Å pore size) (Waters, Milford, MA, USA) where the solvent mixture started at 50:50 methanol : water v/v for 1 min and then was linearly programmed to 100% methanol in 30 min. The third method started with a 50:50 methanol : acetoni trile v/v for 1 min and then linearly increased to 100% acetonitrile in 30 min (same column). For all three methods, the injection volume was 20 µl, the mobile phase flow rate was 0.4 ml/min, solvents were degassed and purged with helium, and the column (when used)
Table 2. Results of Phase I initial toxicity tests of 100% pore waters (salinity adjusted) and whole sediments from New Bedford Harbor. Standard deviation given in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Organism</th>
<th>% Mortality (unless otherwise noted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore water</td>
<td>Amphipod, <em>Ampelisca abdita</em></td>
<td>100 (0)</td>
</tr>
<tr>
<td></td>
<td>Mysid, <em>Mysidopsis bahia</em></td>
<td>90 (14)</td>
</tr>
<tr>
<td></td>
<td>Sea urchin, <em>Arbacia punctulata</em></td>
<td>0% Reduction in fertilization</td>
</tr>
<tr>
<td></td>
<td>Bacterium, Microtox</td>
<td>15% (0) Reduction in luminescence</td>
</tr>
<tr>
<td>Whole sediment</td>
<td>Amphipod, <em>A. abdita</em></td>
<td>100 (0)</td>
</tr>
<tr>
<td></td>
<td>Mysid, <em>M. bahia</em></td>
<td>100 (0)</td>
</tr>
<tr>
<td></td>
<td>Amphipod, <em>Leptochirus plumulosa</em></td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

was conditioned for at least 1 h with a solvent mixture of the initial composition.

Data were collected and analyzed on a PC using HP MS/Chem Station software. Mass spectra of major peaks were compared to spectra of known compounds obtained using this instrument and to reference spectra in the NIST/EPA/NIH Mass Spectral Database for identification.

Single compound tests

Single compound tests were performed on suspected tox-icants. To determine median lethal concentrations (LC50s) of the PCB mixtures Aroclor 1242 and 1254, for *M. bahia* and *A. abdita*, a series of toxicity tests was performed. Aroclors 1242 and 1254 were chosen because they are the major PCB mixtures present in NBH sediments. Three replicates with five organisms/replicate were used. Aroclors in methanol (Ultra Scientific, North Kingstown, RI, USA) were used for the dose-response curves. Dose-response curve concentrations starting at 500 ppb PCBs (50% serial dilution, five concentrations) were conducted. At 48 h the test was renewed with fresh Aroclor solution. Measurements of PCBs were made in the highest and lowest Aroclor concentrations at 48 h before renewal. Concentrations for unmeasured values were estimated by regressing the two measured end members against the nominal concentrations. The tests were terminated at 96 h. Reconstituted seawater controls, and reconstituted seawater–methanol controls were conducted. The LC50s were determined using the measured and regressed concentrations and Spearman–Karber analysis [20].

The LC50 of phthalic acid, bis(2-ethylhexyl) phthalate, was determined by performing tests as described above except test concentrations were measured at 1,000, 333, 111, 37, and 12 ppb.

RESULTS

Phase I TIE

Results of the initial pore-water and whole sediment toxicity tests (Table 2) indicate that the mysid and *A. abdita* were the most sensitive species. All further tests were conducted using these two species. Other test organisms exhibited results ranging between 0 and 15% effect over the control (Table 2).

Results of Phase I TIE baseline and manipulation toxicity tests (Table 3) showed that the C18 SPE was the only manipulation that eliminated toxicity of NBH pore waters. Further, toxicity testing with the C18 column extracts indicated that most of the toxicity was concentrated in the 90% and 95% methanol fractions (Fig. 1a and b). This toxicity was similar in both the

Table 3. Results (% mortality) of New Bedford Harbor (NBH) Phase I baseline and manipulations. Toxicity tests conducted on 100% salinity-adjusted NBH pore water.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Baseline</th>
<th>EDTA a addition</th>
<th>Na2S2O3 addition</th>
<th>Aeration C18</th>
<th>Graduated pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 7</td>
</tr>
<tr>
<td>Amphipod, <em>Ampelisca abdita</em></td>
<td>100</td>
<td>80</td>
<td>100</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Mysid, <em>Mysidopsis bahia</em></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td>0</td>
</tr>
</tbody>
</table>

*EDTA = ethylenediaminetetraacetic acid.*

![Fig. 1. Results of toxicity tests on (a) mysids and (b) *Ampelisca abdita* on C18 fractions with the samples collected from New Bedford Harbor in 1992 and 1994. Unless noted, horizontal axis values are % methanol.](image-url)
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Fig. 2. Gas chromatography–mass spectrometry chromatogram of C₁₈ fraction with suspected and known compounds highlighted.

1992 and the 1994 toxicity tests in that the majority of toxicity was found in the nonpolar fractions. Based on these results, further investigation was focused on the chemical components of the 90% and 95% methanol fractions.

**HPLC fractionation results**

No toxicity was detected in the NBH samples relative to the controls after the 90% and the 95% methanol samples had been fractionated using HPLC.

**GC-MS results**

Initial characterization of both the 90% and 95% methanol samples was performed on GC-MS. A pattern of peaks similar to known PCB patterns was revealed and quantified using GC-ECD (Fig. 2). All major peaks in the PCB area of the total ion chromatogram were verified as PCBs by comparison of spectra to NIST standards, comparison to library spectra, or by the presence of ion clusters characteristic of PCBs (e.g., ion clusters at mass 222, 256, 292, and 326). No PCBs were detected in the operational blank.

The sum of PAH concentrations was 2 and 3 ppb for the 90% and 95% methanol fractions, respectively. No PAHs were detected in the operational blank.

In addition to PCBs and PAHs, two unknown peaks were observed. Peak A was tentatively identified as 1-H-pyrazole and peak B was positively identified as bis(2-ethylhexyl) phthalate (Fig. 2). Both of these compounds were also detected in the operational blanks.

**HPLC-MS results**

The LC-MS analyses were performed in order to detect compounds that would not be able to pass through either the LC column (direct particle beam injection) or a GC column (two C₁₈ solvent gradient methods); however, no compounds different from those found in the GC-MS method were detected.

Direct injection of the toxic 90% and 95% methanol samples into the MS via the particle beam resulted in all compounds eluting in an unresolved series of peaks (<2.0 min) tentatively identified as PCBs, phthalates, and pyrazole. Both solvent gradient methods resulted in later eluting peaks that again contained PCBs, phthalates, and pyrazole.

**GC-ECD results**

The sum of the 18 NOAA Status and Trends PCB congeners was 12 and 27 ppb for the 90% and 95% methanol C₁₈ fractions, respectively. The blank PCB concentration was 0.02 ppb. Bis(2-ethylhexyl) phthalate concentrations were 0.4 and 0.9 ppb for the 90% and 95% methanol fractions, respectively. Instrumental error for these samples was approximately 12%.

**Toxicity test results of single compounds and Aroclors**

Results of 48- and 96-h LC₅₀s for Arochlor 1242 and 1254 are presented in Table 4. Arochlor 1242 was more toxic to both mysids and A. abdita than was Arochlor 1254. Measured values for Arochlor 1242 and 1254 tests were often an order of magnitude below nominal values. All LC₅₀ values were calculated using measured and regressed concentrations. No toxicity was observed in the reconstituted seawater or the reconstituted seawater and methanol controls.

The LC₅₀ for bis(2-ethylhexyl) phthalate was above 1,000 ppb (the highest concentration tested) for both mysids and amphipods. Pryazole was not tested for toxicity because blank concentrations were higher than sample concentrations and no toxicity was observed in the blanks.

**DISCUSSION**

The initial pore-water tests indicated that NBH pore waters were not acutely toxic to all of the tested organisms. Based on these results and the results of other investigators [21,22], we suggest performing assays with more than one species for the screening of any sediment in order to prevent false negative results (type II error).

The combination of no removal of toxicity with ethylenediaminetetraacetic acid (EDTA) and complete removal of porewater toxicity with C₁₈ SPE suggests that organic compounds and not metals were the cause of toxicity in these samples. Although C₁₈ columns are known to remove some metals, particularly Cu [23], because addition of EDTA did not remove toxicity we concluded that metals, although high in some areas of NBH [6], did not contribute to the toxicity of the NBH pore waters examined. Although ammonia has been shown to be a cause of toxicity in some sediments [4,5], the pH manipulation did not indicate the presence of toxic ammonia. The pattern of toxicity associated with ammonia is higher toxicity at pH...
9 (due to higher concentrations of the more toxic, unionized ammonia), and lower toxicity at pH 7 (due to loss of the unionized ammonia). Because we did not observe these results, we concluded that ammonia was not a toxicant in these samples.

Fractionation and testing of the toxicity isolated on the C<sub>18</sub> SPE revealed that toxicity was concentrated in the 90% and 95% methanol fractions. Further fractionation of the 90% and 95% methanol fractions from the C<sub>18</sub> SPE using HPLC resulted in loss or dilution of toxicity. Therefore, when these experiments were repeated in 1992 and then again in 1994, further fractionation of the 90% and 95% methanol fractions using HPLC was not performed. Toxicants quantified in the C<sub>18</sub> SPE 90% and 95% methanol fractions mixtures included PCBs and PAHs. Quantification of PAHs (by GC-MS) indicated the sum of the PAHs to be <2.0 and <3.0 ppb for the 90% and 95% methanol fractions, respectively. The lowest effective concentrations (LOECs) for fluoranthene, pyrene, and acenaphthene for <i>M. bahia</i> and <i>A. abdita</i> range from 30 to 1,100 ppb (measured concentrations) [24,25]. This range is one to three orders of magnitude higher than the sum of the PAHs (which includes fluoranthene, pyrene, acenaphthene, and 12 other PAHs [see Results section]) found in the NBH fraction. Barring a very strong synergistic effect, the PAH concentrations in the 90% and 95% methanol fractions were too low to account for the observed toxicity. Swartz et al. [26] developed a model of additive acute toxicity of PAHs to amphipods, which indicated that a strong synergistic effect of PAHs is unlikely and therefore PAHs are probably not the cause of toxicity in NBH Superfund site sediments. Concentrations of PCBs in the 90% and 95% methanol fractions were within the range of the LC50s of <i>M. bahia</i> and <i>A. abdita</i> (10–109 ppb, Table 4). Our LC50 values were within the range of values for PCBs reported by other investigators [27,28] and indicate that PCBs may be responsible for the acute toxicity in NBH sediments. Although we recognize that some of these values may be above the solubility limit of PCBs (approximately 50 ppb in seawater [29]), measured concentrations may include molecules of PCBs that are not truly dissolved (i.e., associated with colloids or dissolved organic matter present in the reconstituted seawater).

Differences between measured (lower) and nominal Aroclor 1254 and 1242 concentrations in toxicity tests were most likely due to sorption onto surfaces and loss due to volatilization. The order of magnitude difference underlines the importance of measured chemical values for toxicity tests with nonpolar compounds.

Liquid chromatography–mass spectrometry was performed in order to detect compounds that may play a role in contributing to toxicity but are not volatile enough to pass through a GC column. Similarly, direct injection into the LC-MS via the particle beam was performed to detect compounds that would not pass through the LC column. This was of particular concern because the toxicity was lost after passing the C<sub>18</sub> SPE fractions through an LC column earlier in these experiments. The toxicity loss after HPLC fractionation may have been due to the inability of the solvents to elute sorbed compounds from the column, or to dilution of the toxicity response until it was too weak to be detected. With this particular sediment, LC-MS did not detect any compounds not found with GC-MS. In sediments (as opposed to the water column) where more nonpolar, organic compounds would be expected to accumulate, this may be the norm, as GC-MS is generally considered the appropriate instrumentation to detect nonpolar, organic compounds.

Two unknown peaks were identified using GC-MS. (These peaks were also present in the LC-MS runs.) The first, tentatively identified as 1-H-pyrazole, was present in both the 90% and 95% methanol fractions as well as the operational blanks. Comparison of the peak abundance indicated there was more of this compound in the operational blanks than in the sample fractions. Because no toxicity was observed in the operational blank, it can be inferred that the lower concentration of the compound in the sample fraction was not contributing to toxicity. Although the presence of this peak may have been an operational artifact, the toxicity contribution of this compound must be considered in order to accurately assess the toxicity of the fraction. The second compound, bis(2-ethylhexyl) phthalate, was present at higher concentrations in the sample fractions than in the blank. The results of our LC50 tests for this phthalate indicated that this compound was not a toxicant for amphipods and mysids in our pore-water samples. Our results (LC50 > 1,000 ppb) agree with a published value of >370 ppb [30]. The very low concentrations of 0.4 and 0.9 ppb bis(2-ethylhexyl) phthalate in the 90% and 95% methanol fractions, respectively, would not have been responsible for the observed toxicity.

Compounds that may have coeluted with PCBs but were not detectable due to their low concentrations and our chemical methods could include dibenzo-p-dioxins and dibenzofurans. These compounds are of concern because, despite their low concentrations, they have relatively high potencies. Although we have no data for test species used in this study, rainbow trout egg LD50s are 230 to 488 pg/g and 8,000 pg/g for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF), respectively [31]. Pruell et al. [7] reported TCDD present at 0 to 4 pg/g (detection limit 1 pg/g) and TCDF present at 1,180 pg/g in NBH sediments. The TCDD concentrations in NBH were relatively low compared to sediments in Black Rock Harbor, Connecticut, USA; Providence River, Rhode Island, USA and the Hudson River, New York, USA [32]. This is in contrast to relatively high TCDF concentrations in NBH sediments compared to other New England sediments [32].

To estimate pore-water concentrations we used the equation:

\[
\text{Concn. in porewater} = \frac{\text{Concn. in sediment}}{K_p}
\]

where:

\[
K_p = K_{oc} f_{oc}
\]

and \(f_{oc}\) is the fraction of organic carbon in the sediment. Using a \(K_{oc}\) value for dioxin of \(3.3 \times 10^9\) [33], and an \(f_{oc}\) of 18% [7] we calculated pore-water concentrations of TCDD to be \(6.7 \times 10^{-6}\) pg/ml. Assuming the same organic carbon and \(K_{oc}\) value for TCDF we calculated TCDF pore-water concentrations to be \(2 \times 10^{-3}\) pg/ml. These numbers are six to eight orders of magnitude below the LD50 for rainbow trout embryos. Although we understand that there may be wide variations in species sensitivities, and many other congeners of TCDDs and TCDFs in addition to 2,3,7,8-TCDD and 2,3,7,8-TCDF exist, these examples estimate concentrations that may be available to organisms. From this exercise we hypothesize that TCDD and TCDF concentrations were too low to account for much, if any, of the acute toxicity observed.
CONCLUSION

The NBH Superfund site contains toxic sediments, with many potential sources of this toxicity. The overwhelming source of chronic and reproductive toxicity is suspected to be PCBs. Our Phase I TIE results indicated that metals and ammonia were not major contributors to the toxicity of these sediments. Comparison of LC50 data for PAHs and PCBs indicated that PAH concentrations were not high enough to account for the observed sample toxicity; however, PCB sample concentrations were within the range of toxic effects. Two unknown compounds were observed in the blanks and in the toxic fraction. The concentration of one (tentatively identified as a pyrazole) was higher in the nontoxic blank than in the toxic sample; therefore, the toxicity from the suspected pyrazole was discounted. The concentration of the other unknown (positively identified as bis(2-ethylhexyl) phthalate) was three orders of magnitude lower than the LOEC for the compound. Calculations based on literature values of TCDD and TCDF indicated that the concentrations of these compounds in NBH sediments were probably too low to cause the acute toxicity observed in these pore waters. Based on the above evidence, we concluded that PCBs are responsible for acute toxicity to Ampelisca exposed to sediments from the New Bedford Harbor Superfund site. This research demonstrated that chemical fractionation combined with toxicity assays is an effective method of demonstrating causes of toxicity in sediment pore waters and, more specifically, that TIE methods developed for surface waters and effluents are effective tools for evaluating marine pore waters.

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