



Project Summary

Analysis of the NBS Sediment by the MRI Sludge Protocol

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An extraction technique, involving homogenization of a sediment sample with dichloromethane at dual pH and phase separation by centrifugation, was used in the determination of 51 organic priority pollutants as identified in a standard reference sediment sample. These compounds were spiked into the sediment and equilibrated for a defined period of time. The extraction was performed first at $\text{pH} > 11$ to isolate the base/neutral compounds; acidic compounds were extracted at < 2 . The extract containing the base/neutral compounds was fractionated by gel permeation chromatography (GPC). Separation and identification of individual compounds in the silica gel fractions was accomplished by gas chromatography/mass spectrometry (GC/MS). Method precision and accuracy are discussed. Tentative identifications of other organic compounds found in the sediment are given.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

This project investigated the application of the EPA Interim Protocol for the Analysis of Extractable Organic Priority Pollutants in Industrial and Municipal Wastewater Treatment Sludge for isolation of selected test compounds spiked into a sediment sample. The extraction procedure involved homogenization of a sediment/water slurry with dichloromethane at dual pH followed by centrifuga-

tion. A silica gel fractionation of the base/neutral extract and a GPC fractionation of the acidic extract were performed. The compounds selected for this study were 51 priority pollutants representative of nonpolar and polar, basic and acidic, stable and reactive compounds. A standard reference sediment material available from the National Bureau of Standards (NBS) was used as matrix.

Experimental

The standard reference sediment material was purchased from the NBS and is identified as NBS Standard Reference Material No. 1645. According to NBS, the material was dredged from the bottom of the Indiana Harbor Canal near Gary, Indiana. The material was screened to remove foreign objects, freeze dried, and sieved (particle size $< 180 \mu$). The material was sterilized by radiation to minimize biological activity.

The individual stock solutions for each of the 51 compounds (concentration approximately 4 mg/mL) were made in methanol, except for hexachlorobenzene, chrysene and dibenzo(a,h)anthracene stock solutions, which were made in tetrahydrofuran, and anthracene and benzo(a)pyrene, which were made in acetone. Internal standards naphthalene- d_8 , anthracene- d_{10} , and chrysene- d_{12} were purchased from Merck, Sharp and Dohme, Stable Isotope Division (Quebec, Canada).

A 20-g aliquot of the NBS Standard Reference Material was loaded into a 225-mL centrifuge bottle and diluted with 60 mL purified water. The sample was homogenized with Tekmar Tissuemizer blending probe for 30 sec to enhance the

wetting of the sediment. The spike was added immediately following homogenization and allowed to equilibrate with the sediment through continuous mechanical tumbling for 24 hours at 4°C. Three sequential extractions with 80-mL aliquots of dichloromethane were performed first at pH > 11 and then at pH < 2. Following each addition of dichloromethane, the sample was homogenized for 30 sec with a Tekmar Tissuemizer blending probe, and centrifuged for 30 min at 2,500 rpm to promote phase separation. Three distinct layers consisting of an aqueous layer on top, a dark solvent layer in the middle, and a firm solid layer on the bottom were observed. The base/neutral extracts were combined and dried by passage through a short column of anhydrous sodium sulfate prior to concentration to 5 mL in a Kuderna-Danish evaporator. Following extraction at pH < 11, the sediment/water slurry was adjusted to pH < 2 with 6 N hydrochloric acid, and the extraction, extract drying, and concentration steps were repeated to isolate the acidic compounds.

Cleanup of the base/neutral extracts was performed by silica gel chromatography using 3% water deactivated silica (Silica Woelm, 70/150 mesh, ICN). The elution patterns and the recoveries of the 42 base/neutral compounds from the silica gel column were determined in duplicate at two levels (8 µg, 80 µg, per column), corresponding to the spiking levels in sediment samples. The compounds were loaded onto the silica gel column by mixing appropriate aliquots of the stock solution with 2.5g of 3% water-deactivated silica gel in a 10-mL beaker. The solvent was removed under a gentle stream of dry nitrogen, and the adsorbent containing the spikes was transferred to the silica gel column. Four fractions were collected: Fraction I eluted with 100 mL of hexane; Fraction II eluted with 50 mL of 10% dichloromethane in hexane; Fraction III eluted with 50 mL of 50% dichloromethane in hexane; and Fraction IV eluted with 150 mL of 5% acetone in dichloromethane. The sediment extracts were processed identically.

Extracts containing the acidic compounds were fractionated by GPC using Biobeads SX-3 gels (BioRad, Inc.). The GPC column was calibrated using solutions of corn oil, n-butylbenzylphthalate, and phenols (e.g. phenol, 2,4-dichlorophenol, and pentachlorophenol) as described in the test procedure. The eluting solvent was dichloromethane. GPC recoveries of phenols were determined at 8 µg and 80 µg per column, corresponding to the spiking levels of the sediment samples.

A Varian 3700 gas chromatograph equipped with an effluent splitter and a fused silica capillary column (30 m x 0.25 mm ID) coated with SE-54 (film thickness 0.25 µ) was used for screening the various fractions prior to GC/MS analysis. The oven was held at 50°C for 4 min during injection and then temperature programmed at 4°C/min to 300°C (10 min hold) for the base/neutral fractions. The oven was held at 60°C for 5 min and temperature programmed at 8°C/min to 325°C for the acid fractions. A flame ionization detector (FID) and an electron capture detector (ECD) were used simultaneously (effluent split ratio 1:1).

GC/MS analyses were performed on a Finnigan 4021 quadrupole mass spectrometer interfaced to a Finnigan 9600 gas chromatograph and operated under the control of IncoS 2300 data system. All separations were performed by splitless injection using a SE-54 fused silica capillary column (30 m x 0.25 mm ID) connected directly to the ion source. The mass spectrometer was operated in the electron impact mode. Calibration for the mass spectrometer was checked daily using decafluorotriphenylphosphine (DFTPP); resolution; focusing was adjusted to meet the DFTPP criteria. Emission current was in the 0.4-0.6 mA range; ionization energy was eV, and the electron multiplier was operated at 1.7 kV with the preamplifier set at 10⁻⁸A/V.

Results and Discussion

The sample preparation technique described here was originally developed for POTW sludges. In order to be applied to the NBS sediment, an aliquot of the dry sediment is first slurried with water and then extracted with dichloromethane using a high speed homogenizer. If the sediment particles are not within a relatively narrow particle size range (e.g. 20 to 200 µ), difficulties in isolating the organic layer from the aqueous and the solid layers will be experienced.

Although the evaluation experiments were conducted using a set of compounds representative of halogenated hydrocarbons, polycyclic aromatic hydrocarbons, phenols, etc., the results indicate that this extraction technique can be used to isolate other nonpolar and weakly polar compounds.

The cleanup of the base/neutral and the acidic fractions was performed by silica gel and GPC, respectively. Most of the halogenated hydrocarbons (e.g., dichlorobenzenes, trichlorobenzene, hexachlorobutadiene, hexachlorocyclopentadiene, hexachlorobenzene) eluted in Fraction I, whereas the polycyclic

aromatic hydrocarbons (e.g. fluoranthene, pyrene, benzo(a)pyrene, dibenzo(a,h)anthracene) eluted in Fraction III. The more polar compounds such as bis(2-chloroethyl) ether, N-nitrosodi-n-propylamine, bis(2-chloroethoxy)methane, dinitrotoluenes, phthalates) were identified in Fraction IV. Several compounds (e.g., naphthalene, chloronaphthalene, γ-BHC, p,p'-DDE) were found to spread over two or three silica gel fractions; however, for each compound, at least 75% of the amount spiked eluted in only one of the fractions. The data indicated that with a few exceptions, the recoveries of the base/neutral compounds from the cleanup procedures were quantitative and reproducible.

All phenols were recovered from spiked blanks at both spike levels. The chromatographic behavior of dinitrophenols may have precluded their identification at levels below 8 ng/µL in the GPC fractions. Due to these problems, these compounds were not reported for the spiked sediment.

The results of the precision and accuracy determinations for the base/neutral compounds are given in Table 1. Of all recovery determinations in Table 1, 51% fell within the range 60 to 120%. An additional 16% were below 60% recovery, and 8% were above the 120% recovery. Due to the high concentrations of several compounds in the unspiked sediment, their recoveries could not be determined at the lower spike level. For example, fluoranthene, pyrene, chrysene, and benzo(a)pyrene had levels above 10,000 ng/g dry sediment. Furthermore, because of the complexity of the sediment sample, coeluting compounds may have also contributed to the high standard deviations of the recoveries of many of the spikes and the very poor recoveries of several compounds. Polar and reactive compounds such as hexachlorocyclopentadiene, benzidine, 3,3'-dichlorobenzidine, heptachlor epoxide (only at 400 ng/g dry sediment), endrin, and α-endosulfan were not recovered.

The results of the precision and accuracy determinations for the acidic compounds are given in Table 2. Of all recovery determinations, 64% were greater than 70%, 29% were between 40% and 70%, and 7% were zero. Quantitative recoveries (>70%) were found at both spike levels for phenol, 2-chlorophenol, 2,4,-dichlorophenol, 2,4-dimethylphenol, and pentachlorophenol. 4-Nitrophenol was not detected at 400 ng/g; however, its recovery was satisfactory at the higher spike level. The chromatographic behavior of this compound

Table 1. Results of the Precision and Accuracy Evaluations for the Sludge Protocol Applied to the Analysis of Base/Neutral/Pesticide Compounds in the Spiked NBS Sediment

Compound No.	Compound	Unspiked sediment ^a concentration (ng/g dry sediment)	% Recovery ^a	
			Spike level 400 ng/g dry sediment	Spike level 4,000 ng/g dry sediment
1	Bis(2-chloroethyl) ether	ND	96 ± 23	112 ± 19
2	1,3-Dichlorobenzene	ND	63 ± 9	68 ± 16
3	1,2-Dichlorobenzene	ND	66 ± 14	75 ± 22
4	Hexachloroethane	ND	41 ± 15	62 ± 5
5	N-Nitroso-di-n-propylamine	ND	95 ± 38	89 ± 11
6	Nitrobenzene	ND	43 ± 3	102 ± 26
7	Bis(2-chloroethoxy)methane	ND	94 ± 39	81 ± 11
8	1,2,4-Trichlorobenzene	ND	67 ± 4	58 ± 13
9	Naphthalene	880 ± 850	64 ^c	70 ± 35
10	Hexachlorobutadiene	ND	63 ± 2	71 ± 17
11	Hexachlorocyclopentadiene	ND	0	0
12	2-Chloronaphthalene	ND	72 ± 10	94 ± 20
13	Acenaphthylene	1,820 ± 350	d	86 ± 24
14	2,6-Dinitrotoluene	ND	110 ± 51	102 ± 36
15	Acenaphthene	850 ± 120	d	104 ± 24
16	2,4-Dinitrotoluene	ND	49 ^c	64 ± 50
17	Fluorene	1,580 ± 130	d	82 ± 7
18	4-Chlorophenyl phenyl ether	ND	86 ± 42	74 ± 7
19	Diethylphthalate	ND	33 ± 14	109 ± 13
20	4-Bromophenyl phenyl ether	ND	91 ± 50	89 ± 2
21	Hexachlorobenzene	ND	46 ± 10	53 ± 4
22	Phenanthrene	3,770 ± 300	d	83 ± 26
23	Anthracene	2,090 ± 72	d	25 ± 11
24	Di-n-butylphthalate	580 ± 65	156 ^c	100, 137
25	Fluoranthene	14,760 ± 2,810	d	174 ± 78
26	Pyrene	20,020 ± 4,630	d	148, 252
27	Benzidine ^b	ND	0	0
28	Endosulfan sulfate	1,160, 455	97, 137	0
29	n-Butylbenzylphthalate	920	d	103 ^c
30	Chrysene	27,000 ± 6,500	d	d
31	3,3'-Dichlorobenzidine	ND	0	0
32	Bis(2-ethylhexyl)phthalate	7,560, 10,790	d	d
33	Benzo(a)pyrene	22,850 ± 6,200	d	128 ± 23
34	Dibenzo(a,h)anthracene	5,960 ± 420	d	113 ± 42
35	γ-BHC	3,050 ± 1,270	d	50 ± 19
36	Aldrin	ND	44 ^c	22 ± 13
37	Heptachlorepoxyde	ND	0	54 ± 9
38	p,p'-DDE	340 ± 100	41 ^c	76 ± 32
39	Endrin	ND	0	0
40	p,p'-DDD	120	121, 186	104 ± 18
41	p,p'-DDT	840 ± 20	d	155 ± 43
42	α-Endosulfan	ND	0	0

ND = Not detected.

^a Mean ± standard deviation for three determinations.

^b Benzidine not detected by GC/MS in any of the standards at concentrations ≤ 80 ng/μl.

^c Single determination reported; levels in the other replicates were below the unspiked sediment level.

^d Concentrations determined in the spiked sediment were below the unspiked sediment level.

may have precluded its identification at low spike levels.

The GC/MS analyses of the silica gel fractions and the GPC fraction generated from the NBS sediment extract resulted in the identification of over 100 base/neutral compounds and 11 acidic compounds, without considering the various isomers of many of these compounds.

Because authentic reference standards were not analyzed for each compound, the identifications are tentative (a few compound identifications were confirmed with standards). Most of the compounds

give mass spectra that matched the mass spectra in the NBS library. Identification of specific positional isomers (e.g. dimethylnaphthalenes, trimethylnaphthalenes, etc.) could not be obtained from the mass spectrometric information.

Whenever an identification was made, either by library search routines or by manual interpretation, mass chromatograms for the most intense ions in the mass spectrum were obtained. In many instances, this allowed identification of other isomers and assignment of coeluting peaks. Although in many cases the

library search did indicate the positional isomers that best match a particular unknown mass spectrum, this was not sufficient for confirming the compound identity. Consequently, no identification has been made about the position of the substituent or the particular alkyl radical.

Fractionation of the base/neutral extract by silica gel chromatography reduced sample complexity and, consequently, made possible the identification of minor components in the sample. Furthermore, the chromatographic behavior of several classes of compounds (e.g.,

Table 2. Results of the Precision and Accuracy Evaluations for the Sludge Protocol Applied to the Analysis of Acidic Compounds in the Spiked NBS Sediment

Compound No.	Compound	Unspiked sediment concentration (ng/g dry sediment)	% Recovery	
			Spike level 400 ng/g dry sediment	Spike level 4,000 ng/g dry sediment
1	Phenol	1,810, 2,005	112, 128	78 ± 21
2	2-Chlorophenol	ND	106 ± 11	78 ± 18
3	2,4-Dimethylphenol	ND	99 ± 12	55 ± 3
4	2,4-Dichlorophenol	ND	99 ± 16	76 ± 5
5	2,4,6-Trichlorophenol	ND	67 ± 8	63 ± 10
6	2,4-Dinitrophenol	ND	0	0
7	4-Nitrophenol	ND	0	46 ± 11
8	4,6-Dinitrocresol	ND	0	0
9	Pentachlorophenol	ND	95 ± 2	71 ± 3

ND = Not detected.

^a - % Recovery determined from triplicate measurements;

$$\text{Recovery} = \frac{\text{Spiked sed. conc} - \text{unspiked sed. conc}}{\text{Spike added}}$$

sulfur containing heterocyclics, aldehydes keytones) on silica gel helped in the assignment of the identifications.

For example, polar compounds such as benzofluorenone and methylbenzofluorenone (m/z 230) was identified by reverse library search in Fraction IV; however, no mass spectral match was found for the mass spectrum with a parent ion at m/z 244. Assigning a mass spectrum with a parent ion at m/z 244 methylbenzofluorenone was based on two considerations: similar mass spectral fragmentation pattern with benzofluorenone and similar chromatographic behavior on silica gel of benzofluorenone and methylbenzofluorenone.

The GC/MS chromatograms showed that most of the compounds of environmental significance elute in Fractions II and III. Fraction I contains mostly saturated hydrocarbons (alkanes) that were not resolved under the conditions used. Similarly, Fraction IV contains polar compounds (naturally occurring materials) that would interfere in the analysis, had the sample fractionation not been performed.

Summary

Given the complexity of the matrix, the precision and accuracy results presented here demonstrate that the extraction method, involving homogenization of the wet sediment with dichloromethane followed by centrifugation, can be applied with minor modifications to the analysis of sediments. Although these experiments were conducted using selected compounds, the results indicate that other nonpolar or weakly polar compounds can also be isolated. In fact, the qualitative information presented here does indicate that a large number of polycyclic aromatic hydrocarbons and sulfur-containing heterocyclics were isolated from the sediment matrix.

Polar compounds or reactive compounds containing nitro or epoxy groups were not amenable to the analysis procedures chosen for evaluation.

Fractionation of the sediment extract containing the base/neutral compounds by silica gel chromatography and of the acidic extract by GPC helped reduce the sample complexity, thus improving the quantitation of the priority pollutants without any significant effect on the analyte recovery for many of the compounds.

Analysis of the NBS sediment extracts containing the base/neutral compounds and the acidic compounds was successfully performed using a fused silica capillary column coated with SE-54. The

inertness and excellent resolution of the capillary column allowed the detection of nanogram levels of phenols in the presence of high levels of interfering coextractants.

Tentative identifications were provided for the organic compounds extracted for sediment by the extraction and analysis procedures described in this paper. Most of the compounds identified in the silica gel and GPC fractions were polycyclic aromatic hydrocarbons, alkyl substituted polyaromatics, sulfur-containing heterocyclics, alkyl substituted sulfur-containing heterocyclics, and several polycyclic ketones.

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Stephen Billets was the EPA Project Officer (see below for present contact).

The complete report, entitled "Analysis of the NBS Sediment by the MRI Sludge Protocol," (Order No. PB 84-133 750; Cost: \$16.00, subject to change) will be available only from:

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