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Microwave-Assisted Extraction of Organic Compounds from Standard Reference Soils and Sediments

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As part of an ongoing evaluation of new sample preparation techniques by the U.S. Environmental Protection Agency (EPA), especially those that minimize waste solvents, microwave-assisted extraction (MAE) of organic compounds from solid materials (or "matrices") was evaluated. Six certified reference materials containing polynuclear aromatic hydrocarbons (PAHs) and a few base/neutral/acidic compounds, all of which are common pollutants of interest to the EPA, were subjected to MAE in a closed-vessel microwave system with hexane/acetone (1:1) at different temperatures (80, 115, and 145 °C) and for different periods of time (5, 10, and 20 min). For comparison, the same samples were subjected to room-temperature extraction by allowing the solvent mixture to stay in contact with the solid matrix the same amount of time as the microwave-extracted sample (including any cooling time). Whereas the average recovery at room temperature was ~52%, the MAE recoveries for the 17 PAHs (3 of which were deuterated PAHs that were spiked into these matrices) from the six matrices were 70% at 80 °C, 75% at 115 °C, and 75% at 145 °C. Although the average recoveries increased slightly with extraction time, the increase was not statistically significant. The performance of the technique varied with the matrix and the analyte. Eleven PAHs had average recoveries in the 65–85% range, and three compounds (acenaphthene, benzo[a]pyrene, and fluorene) had recoveries of ~50%. The spiked-compound recoveries were 77% for acenaphthene-d_{10}, 105% for fluoranthene-d_{10}, and 85% for benzo[a]anthracene-d_{12}. Experiments with 14 phenols and 20 organochlorine pesticides indicated that MAE is a viable alternative to the conventional Soxhlet/Soxtec and sonication techniques. The MAE technique requires smaller amounts of organic solvents, and sample throughput is increased by shorter extraction times (10 min) and by simultaneous extraction of up to 12 samples.

Use of microwave energy to enhance extraction of organic compounds from solid matrices such as soil, seeds, foods, and feeds was reported by Ganzler and Salgo in two publications in 1986 and 1987.[1,2] These researchers used a conventional, household microwave oven to irradiate solvent/sample suspensions for 30 s up to seven times each. They reported that the microwave-assisted extraction (MAE) method was more efficient than Soxhlet extraction for polar compounds.[1] Recently, Onuska and Terry[3] used microwave energy to extract organochlorine pesticides from sediment samples; they reported quantitative recoveries and no compound breakdown due to sample exposure to microwaves. Extraction of essential oils and other oils from biological materials such as plant and fish tissue by exposure to microwave energy was recently described in a patent application.[4] In a U.S. patent,[5] extraction of natural products from mint, sea parsley, cedar foliage, and garlic with hexane, methylene chloride, or ethanol in two or more stages is described. Other researchers have reported use of microwave energy to extract stabilizers from polyolefins.[6]

As part of an ongoing U.S. Environmental Protection Agency (EPA) program addressing sample preparation techniques that prevent or minimize pollution in analytical laboratories, this study addresses the extraction of organic compounds using a closed-vessel MAE technique. Six certified reference soil and sediment materials containing PAHs and a few base/neutral/acidic compounds of interest to the EPA were extracted with hexane/acetone (1:1) at different temperatures and for different periods of time to establish whether this technology has merit. Comparative measurements were made using conventional extraction techniques (e.g., Soxhlet/Soxtec and sonication extraction), room-temperature extraction (as defined later), and MAE. The results indicated that MAE could be a viable alternative to the conventional Soxhlet/Soxtec and sonication methods. This technique uses smaller amounts of organic solvents, and sample throughput is increased by reduced extraction time (10 min) and by the capability of extracting up to 12 samples simultaneously (this study was performed with six samples being extracted simultaneously).

EXPERIMENTAL SECTION

Standards. Analytical reference standards of 14 nonlabeled and three labeled polynuclear aromatic hydrocarbons (PAHs), 14 phenols, and 20 organochlorine pesticides (Table 1) were purchased as composite solutions (concentration 2 mg/mL per compound) from Supelco, Inc. (Bellefonte, PA). The three deuterated PAHs were purchased as neat materials from Cambridge Isotope Laboratory (Woburn, MA). The other

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(2) Ganzler, K.; Salgo, A. *Z. Unters. Forsch.* **1987**, *184*, 274–276.
(3) Onuska, F. E.; Terry, K. A. *Chromatographia* **1993**, *36*, 191–194.
(4) Paré, J. R. J. Eur. Pat. Appl. EP 485668 A1, 1992; *Chem. Abstr.* **1992**, *117* (16), 157431y.
(5) Paré, J. R. J.; et al. U.S. Patent 5,002,784, 1991.
(6) Freitag, W.; John, O. *Angew. Makromol. Chem.* **1990**, *175*, 181–185.

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Table 1. Compounds Investigated in This Study

	compound name		compound name
	PAHs		
1	acenaphthene	10	chrysene
2	acenaphthylene	11	fluoranthene-d_{10}
3	anthracene	12	fluorene
4	anthracene-d_{10}	13	fluoranthene
5	benz[*a*]anthracene	14	indeno(1,2,3-*cd*)pyrene
6	benz[*a*]anthracene-d_{12}	15	naphthalene
7	benzo[*a*]pyrene	16	phenanthrene
8	benzo[*b*+*k*]fluoranthene	17	pyrene
9	benzo[*ghi*]perylene		
	Base/Neutral Compounds		
18	dibenzofuran	25	9*H*-carbazole
19	1,2-dichlorobenzene	26	di-*n*-butyl phthalate
20	1,3-dichlorobenzene	27	bis(2-ethylhexyl) phthalate
21	*N*-nitroso-di-*n*-propylamine	28	isophorone
22	nitrobenzene	29	4-chlorophenyl phenyl ether
23	1,2,4-trichlorobenzene	30	butyl benzyl phthalate
24	2,4-dinitrotoluene		
	Phenols		
31	phenol	38	4-chloro-3-methylphenol
32	2-chlorophenol	39	2,4,6-trichlorophenol
33	2-methylphenol	40	2,4-dinitrophenol
34	3-methylphenol	41	4-nitrophenol
35	2-nitrophenol	42	2,3,4,5-tetrachlorophenol
36	2,4-dimethylphenol	43	2-methyl-4,6-dinitrophenol
37	2,4-dichlorophenol	44	pentachlorophenol
	Organochlorine Pesticides		
45	α-BHC	55	dieldrin
46	β-BHC	56	4,4′-DDE
47	γ-BHC	57	endrin
48	δ-BHC	58	endosulfan-II
49	heptachlor	59	4,4′-DDD
50	aldrin	60	endrin aldehyde
51	heptachlor epoxide	61	endosulfan sulfate
52	γ-chlordane	62	4,4′-DDT
53	endosulfan-I	63	endrin ketone
54	α-chlordane	64	methoxychlor

13 compounds (base/neutral) in Table 1 (except dibenzofuran, which was bought from Supelco as neat material) were purchased as individual stock solutions from ChemService (West Chester, PA) and Supelco, Inc., and were combined with the PAH stock solution to make the working calibration standards for the GC/MS analysis. Dibenzofuran was dissolved separately in methanol at 5 mg/mL and was combined with the PAH stock solution to make the working calibration standards. The purities of all compounds were stated to be higher than 96%. The spiking solution and the working calibration standards were prepared by serial dilution of the composite stock solution containing either the phenols, organochlorine pesticides, PAHs, or base/neutral compounds; for the analysis of the ERA soil samples (defined below), the calibration standards contained the PAHs, the base/neutral compounds, and selected phenols.

Standard Reference Materials. Six certified reference marine sediments and soils were used in this study. HS-3, HS-4, and HS-5 are marine sediments collected from three harbors in Nova Scotia. The materials were purchased from the National Research Council of Canada, Atlantic Research Laboratory (Halifax, NS). According to the certificate of analysis, these materials were freeze-dried, sieved to pass a 125-μm sieve, homogenized in a cement mixer, and then subsampled into 200-g portions. The SRS1941 marine sediment, purchased from NIST (Gaithersburg, MD), is a sediment collected from the Chesapeake Bay at the mouth of the Baltimore Harbor. According to NIST, this sediment was air-dried, pulverized, sieved (<150 μm), homogenized, and subsampled into 70-g portions. The bottled samples were sterilized by ^{60}Co radiation. Additional information about this sediment is reported on the NIST Certificate of Analysis. The SRS103-100 certified reference material is a soil contaminated with PAHs (natural material) and was purchased from Fisher Scientific (Fair Lawn, NJ); the material was prepared by RT Corp. in Laramie, WY. The certified values of the PAHs found in this material are included in the tables in the Results section. The ERA soils (Lots No. 321 and 323) are spiked materials and were purchased from Environmental Resource Associates (Arvada, CO). The certified values for these materials are included in the tables in the Results section.

The soil used in the preliminary experiments was a sandy loam soil (0.1% organic matter) and was obtained from Sandoz Crop Protection (Gilroy, CA).

Solvents. All solvents used in this study were distilled-in-glass, pesticide grade. The solvent mixture chosen for the MAE was hexane/acetone (1:1). Other solvents evaluated for the MAE include tetrachloroethylene, methylene chloride/acetone (1:1), toluene/methanol (10:1), methylene chloride, and toluene/methanol (1:10). The solvent mixture used for Soxtec extraction was hexane/acetone (1:1), and that for sonication extraction was methylene chloride/acetone (9:1).

Microwave-Assisted Extraction Procedure. A 5-g portion of each matrix was accurately weighed into an aluminum dish and was transferred quantitatively to the Teflon-lined extraction vessel. To prepare the wet samples, the calculated volume of water was added and allowed to equilibrate with the matrix for ~10 min. A solution containing the three deuterated PAHs was added to each matrix immediately before adding the hexane/acetone (1:1) solvent (30 mL). The extraction vessel was closed, after ensuring that a new rupture membrane was used for each extraction. Extractions were performed at 80, 115, or 145 °C for 5, 10, or 20 min at 50% power. After extraction, the vessels were allowed to cool to room temperature before they were opened. The supernatant was first filtered through precleaned glass wool and was then combined with the 2–3-mL hexane/acetone rinse of the Teflon extraction vessel containing the soil; the extract was concentrated to ~5 mL using nitrogen blowdown evaporation and was centrifuged twice for 10 min at 2300 rpm to separate the fine particulates. The extract was finally either concentrated to 1 mL or diluted for GC/MS analysis.

All MAEs were performed with a 950-W MDS-2000 microwave sample preparation system (CEM Corp., Matthews, NC) shown in Figure 1. This system was equipped with an inboard pressure and Fluoroptic temperature control system for regulating sample extraction conditions via magnetron power output control. Temperature and pressure control set points could be programmed in five separate heating stages. The instrument controlled either pressure or temperature, depending on which parameter reached its control set point first. Lined digestion vessels (110-mL volume) were used for extractions. The turntable shown in Figure 2 contained a control vessel and 11 standard vessels. The control vessel is illustrated in Figure 3a. The outer body and cap

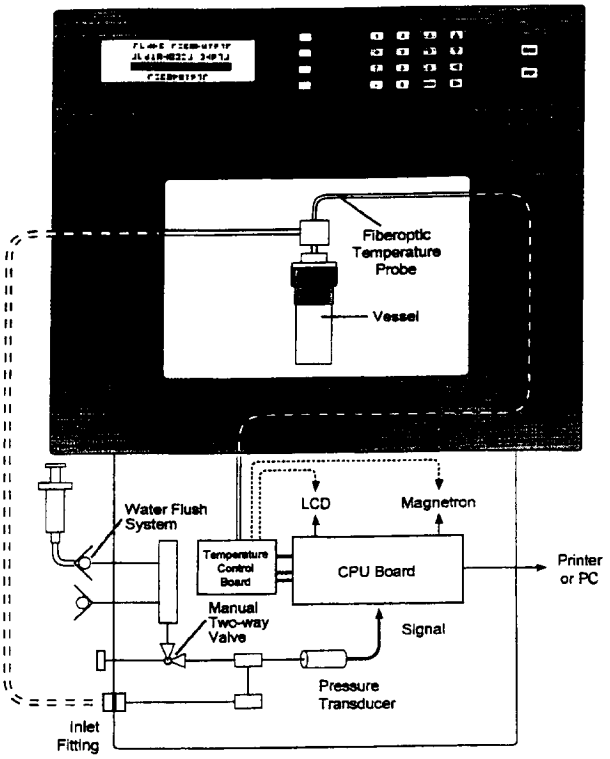

Figure 1. Schematic diagram of the temperature/pressure control system for the MDS-2000 microwave system.

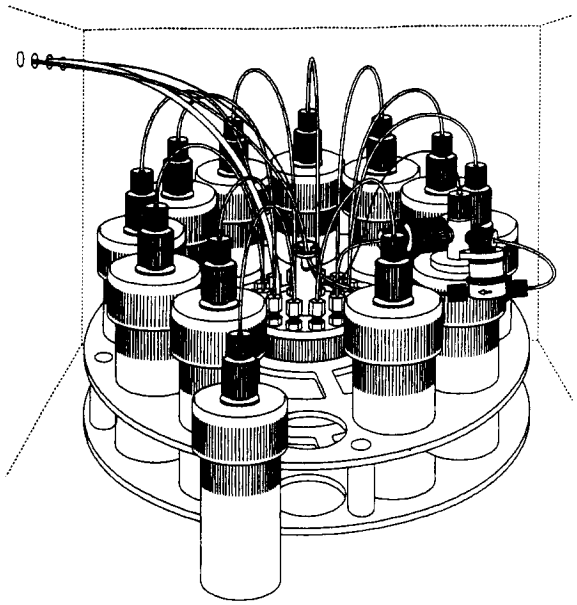

Figure 2. View of the 12 lined digestion vessels, containment vessel, and temperature and pressure probes.

consisted of microwave-transparent Ultem poly(ether imide). The removable inner liner, the liner cover, and the safety rupture membrane were made of Teflon PFA. Gases could escape through the exhaust port if the safety rupture membrane broke or if the vessel were hand-vented by turning the vent fitting. The liner cover of the control vessel had Teflon PFA fittings to allow for pressure tubing connection and for insertion of a Pyrex tube that ran through the cap into the vessel and ended close to the bottom of the vessel. This Pyrex tube, which housed the Fluoroptic probe, provided a seal in the cap and protected the Fluoroptic probe from solvent attack. The standard vessels, one of which is shown in Figure 3b, did not have temperature and pressure ports but had only a rupture membrane and vent stem. These vessel assemblies were rated for operation up to 175 psi and 200 °C.

All vessels shown in Figure 2 were connected to a containment vessel (shown in the center of Figure 2) via $^1/_8$-in. Teflon tubes. The control vessel's temperature and pressure were monitored for control purposes. The Fluoroptic probe extended down through the center of the turntable and was inserted into the control vessel. The $^1/_4$-in. diameter Teflon tube from the sealed containment vessel passed through the oven cavity wall to provide a safe exhaust of any solvent vapor in case the rupture membrane broke.

Other Extraction Procedures. Extractions using a Soxtec apparatus (Tecator, Silver Springs, MD) were performed by extracting 10-g portions of the reference materials with 50 mL of hexane/acetone (1:1); the immersion time and the extraction time were 45 min each. The solvent was evaporated directly in the Soxtec apparatus. Details of this procedure can be found elsewhere.[7,8]

Extractions using a sonic probe (Sonifier 450, Branson Ultrasonics, Danbury, CT), were performed using 5-g portions of each reference material (except for NIST 1941 material, where 3.4 g were used). The solvent/soil suspensions were sonicated for 3 min at 50% power (output setting 3.5), once with 20 mL of methylene chloride/acetone (9:1) and then with 10 mL of methylene chloride/acetone (9:1). The extracts were combined, the solvent was exchanged to hexane, and the hexane solution was concentrated to 1 mL. A silica gel procedure using 1.8 g of silica gel (100–200 mesh, EM Science, Gibbstown, NJ), activated for 16 h at 130 °C prior to use, was used to clean up the extracts. The first fraction that was eluted with 10 mL of hexane was discarded. PAHs were recovered from the silica gel column with 10 mL of hexane/methylene chloride (60:40).

The room-temperature extractions of the certified reference soils and sediments were performed by allowing the solvent mixture to stay in contact with the solid matrix for the same amount of time that was required for the temperature in the microwave-heated vessel (i) to reach the set point, (ii) to be maintained at the set point (5, 10, or 20 min), and then (iii) to cool to room temperature.

Analysis of Extracts. Analyses of the extracts of certified reference soils and sediments and those containing PAHs and selected base/neutral compounds were performed on a 5890 Series II gas chromatograph interfaced to an HP 5971A mass spectrometer MSD/DOS Chemstation (Hewlett Packard, Palo Alto, CA) and equipped with a 5973A autoinjector. Samples were introduced via a 30-m length × 0.25-mm i.d. × 0.25-μm film thickness PTE-5 fused-silica open tubular column (Supelco, Inc.) with helium carrier gas at a linear velocity of 39 cm/s. The column temperature was held at 75 °C for 3 min and then programmed to a final temperature of 300 °C at 12 °C/min, where it was held for 13 min. The injection volume was 2 μL, and the injector temperature was 250 °C. The injector was set in the splitless mode for 1 min after the injection. The electron energy was set at 70 eV, and the electron multiplier voltage was set at 2160 V. Spectral

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(8) Lopez-Avila, V. EPA Report 600/X-91/140, Environmental Monitoring Systems Laboratory, Las Vegas, NV, October 1991.

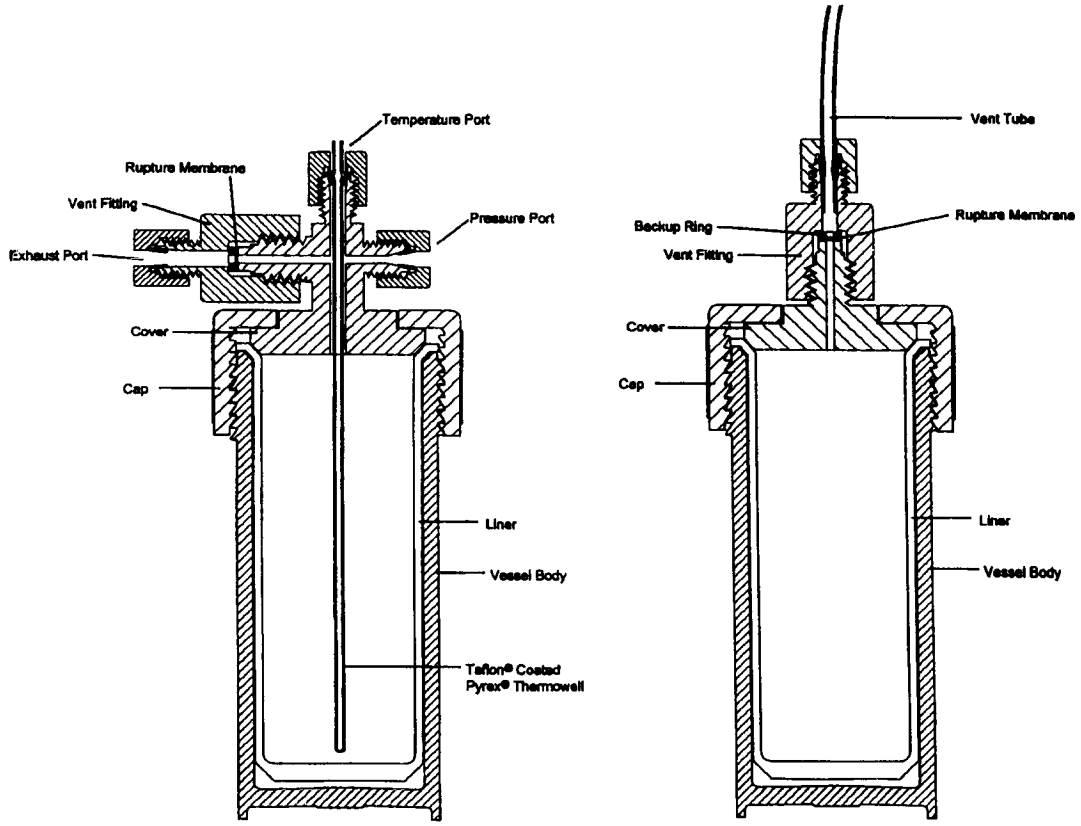

Figure 3. (a) Lined digestion vessel with temperature and pressure control. (b) Lined digestion vessel without temperature and pressure control.

data were acquired at a rate of 1.2 s/scan (scanning range was 40–500 amu). The instrument was tuned daily with PFTBA introduced via the calibration gas valve; the ion intensity was verified using DFTPP introduced via the GC inlet. A five-point internal standard calibration was performed initially to establish the GC/MS linear range. Six internal standards including 1,4-dichlorobenzene-d_4 (IS-1), naphthalene-d_8 (IS-2), acenaphthene-d_{10} (IS-3), phenanthrene-d_{10} (IS-4), chrysene-d_{12} (IS-5), and perylene-d_{12} (IS-6) were spiked into every extract that was analyzed by GC/MS.

Analyses of the extracts containing the 14 phenols or the 20 organochlorine pesticides were performed by gas chromatography with flame ionization detection (FID) or electron capture detection (ECD), respectively. For phenol analysis, we used a 5890 Series II gas chromatograph equipped with an FID and a 5973A autoinjector. Samples were introduced via a 15-m length × 0.53-mm i.d. × 0.88-μm film thickness HP-5 fused-silica open tubular column (Hewlett Packard) with helium carrier gas at a flow rate of 7.1 mL/min. The column temperature was held at 65 °C for 3 min and then programmed to 185 °C (1-min hold) at 10 °C/min and then to 275 °C (5-min hold) at 30 °C/min. The injection volume was 2 μL, and the injector temperature was 200 °C.

For organochlorine pesticide analysis, we used a 5890 Series II gas chromatograph equipped with an ECD and a 5973A autoinjector. Samples were introduced via a 15-m length × 0.53-mm i.d. × 0.88-μm film thickness HP-5 fused-silica open tubular column (Hewlett Packard) with helium carrier gas at a flow rate of 7.5 mL/min. The column temperature was held at 150 °C for 0.5 min and then programmed to 275 °C at 5 °C/min. The injection volume was 1 μL, and the injector temperature was 200 °C.

Safety. The microwave unit should be operated in accordance with CEM's recommended operating safety instructions. The MDS-2000 microwave unit used in this study was recently slightly modified by CEM Corp. to incorporate additional safety features: (a) a Teflon sealing, held in place with polypropylene clips, was mounted in the cavity underneath the mode stirrer so that any sparks from the stirrer would not ignite any organic vapor that might leak into the cavity; (b) an air flow sensor was installed in the exhaust line from the microwave unit (should the sensor detect a decrease in the air flow, then the microwave energy would be shut off); (c) the maximum operating pressure setting was limited to 150 psi; and (d) the lined digestion vessels were modified so that, in case of membrane rupture, the solvent vapors would be retained in a containment vessel and would not escape into the cavity.

RESULTS AND DISCUSSION

Preliminary Experiments. Pure hydrocarbon solvents (e.g., hexane) do not absorb microwave energy. Therefore, a percentage (>10%) of a polar component (e.g., acetone) must be added. Several solvents and solvent mixtures that are commonly used in conventional extraction techniques were tried in the microwave system using the control vessel to establish the time required to reach the maximum temperature. Methylene chloride/acetone (1:1), hexane/acetone (1:1), and toluene/methanol (1:10) reached maximum temperature within 1–3 min (Table 2). We chose hexane/acetone (1:1) in subsequent experiments since this solvent mixture was compatible with electron capture detection. Next, we investigated the effect of solvent volume by varying the volume of hexane/acetone (1:1) from 5 to 30 mL. The results indicated

Table 2. Time Required To Reach the Maximum Temperature under Various Extraction Conditions

solvent type	solvent vol (mL)	matrix type	sample wt (g)	no. of vessels	time to max temp (min)	T_{max} (°C)
		Effect of Solvent				
tetrachloroethylene	10, 20, 30	solvent only	0	1	[a]	
methylene chloride/acetone (1:1)	30	solvent only	0	1	1:15	160–161
hexane/acetone (1:1)	30	solvent only	0	1	2:30	157–159
toluene/methanol (10:1)	30	solvent only	0	1	13:45	110–112
methylene chloride	30	solvent only	0	1	9:45	135–136
toluene/methanol (1:10)	30	solvent only	0	1	1:15	146–147
		Effect of Solvent Volume				
hexane/acetone (1:1)	5	solvent only	0	1	17:00	146
hexane/acetone (1:1)	10	solvent only	0	1	7:45	147–148
hexane/acetone (1:1)	15	solvent only	0	1	7:30	145
hexane/acetone (1:1)	20	solvent only	0	1	5:00	145
hexane/acetone (1:1)	30	solvent only	0	1	2:30	156–160
		Effect of Matrix				
hexane/acetone (1:1)	30	sand	10	1	2:30	156–157
hexane/acetone (1:1)	30	sediment	5	1	2:00	154–155
hexane/acetone (1:1)	30	sandy loam	10	1	1:45	152–153
hexane/acetone (1:1)	30	clay loam	6	1	2:15	151–152
		Effect of Solvent Volume and Sample Weight				
hexane/acetone (1:1)	10	clay loam	2	1	14:00	155
hexane/acetone (1:1)	15	clay loam	3	1	8:30	153
hexane/acetone (1:1)	20	clay loam	4	1	4:45	152
hexane/acetone (1:1)	30	clay loam	6	1	2:15	151
		Effect of Number of Vessels				
hexane/acetone (1:1)	30	solvent only	0	1	2:30	156–160
hexane/acetone (1:1)	30	solvent only	0	6	5:00	156–160
hexane/acetone (1:1)	30	solvent only	0	12	9:30	156–160
		Effect of Sample Moisture				
hexane/acetone (1:1)	30	sand (10% water)	5	1	4:30	142–143
hexane/acetone (1:1)	30	sand (30% water)	5	1	3:30	137–138

[a] Did not heat above 70 °C.

that a 30-mL volume was desirable since it could reach the maximum temperature (156–160 °C) within 2.5 min (Table 2). When various solid matrices were added, the effect of the matrix appeared to be insignificant; the time required for the temperature to reach the maximum temperature was still ~2 min (Table 2).

When varying both the solvent volume and the mass of the sample, while keeping their ratio constant, we found that a 30-mL volume and a 6-g sample gave the shortest heating time (Table 2). When the water content of the sample was adjusted to 10 or 30%, the time required to reach the maximum temperature almost doubled, and the T_{max} was ~15 °C lower than in the case of the dry matrix. When we performed experiments with 6 and 12 vessels, the time required to heat the vessels increased to 5 min for 6 vessels and to 9.5 min for 12 vessels (Table 2).

Sand freshly spiked with seven of the organochlorine pesticides listed in Table 1 was extracted for 5, 10, and 20 min using microwave energy; another spiked sand sample was left in contact with the solvent at room temperature (no microwave energy) for ~5 min. Recoveries were all above 95%, but we could not draw any conclusions about how well the technique performed because simple soaking of the sample with solvent also resulted in quantitative recoveries. The results indicated, however, that the spiked compounds did not degrade when the microwave energy was applied. The recoveries ranged from 95 to 134%; the high values may have been due to slight concentration of the solvent upon opening the vessel too soon after the extraction. In subsequent experiments, we measured the volume of the solvent after extraction and found no changes in the solvent volume when we extracted dry matrices.

Extraction of PAHs from Standard Reference Materials Using Microwave Energy. Four standard reference marine sediments (HS-3, HS-4, HS-5, and NIST SRM1941) and two certified soils (SRS103-100 and ERA, Lot No. 321) were subjected to room-temperature extraction and to MAE with hexane/acetone (1:1) for 5, 10, or 20 min after reaching the set temperature (80, 115, and 145 °C). Three deuterated compounds were spiked into these matrices before extraction at levels comparable to those of their undeuterated counterparts. This allowed us to compare the recoveries of the native and the spiked compounds.

The recovery data for 17 compounds listed under PAHs in Table 1 (14 of which were identified as native compounds and three as spikes) are summarized in Figures 4–7. These recoveries were determined from the values obtained for the extracts by GC/MS and from the certified values provided by the manufacturers. Not all reference materials contained the 17 compounds; therefore, the summarized recovery data pertain to recoveries from four to six matrices, depending on the compound.

Figure 4 shows the average recoveries and the 95% confidence intervals as a function of temperature across the 17 compounds and the six matrices. The average recovery at room temperature was ~52%, which was significantly lower than the average recoveries achieved at 80, 115, and 145 °C (70, 75.5, and 75%, respectively). Since the average recoveries at 115 and 145 °C were almost identical, the experiments to

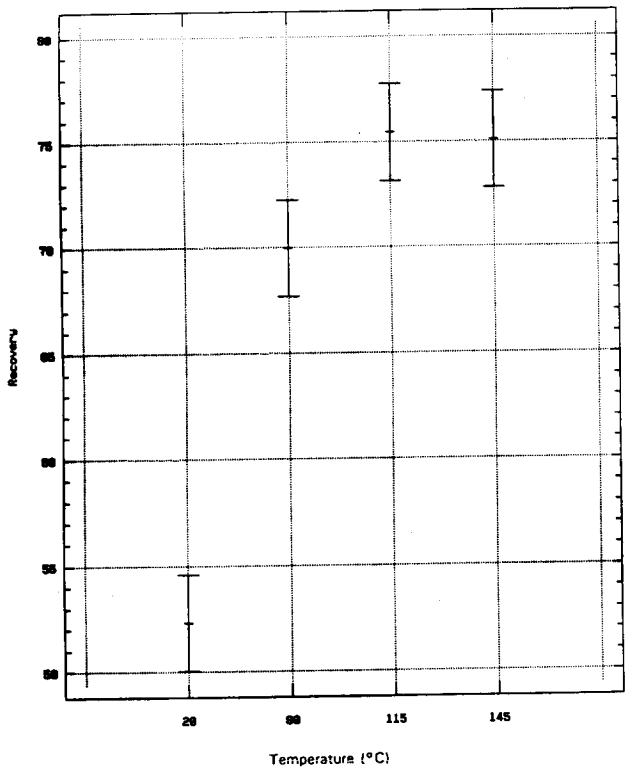

Figure 4. Recovery as a function of temperature for the 17 compounds (four to six matrices): 95% LSD intervals for factor means.

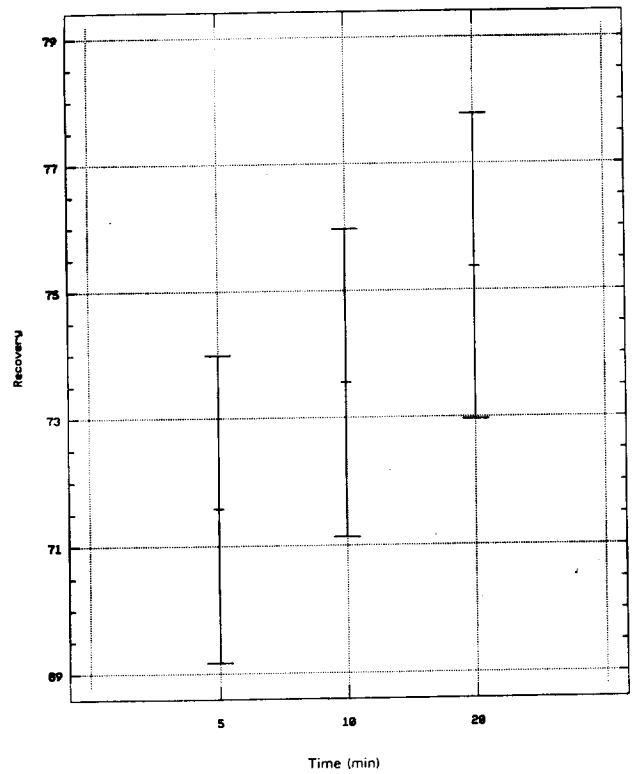

Figure 5. Recovery as a function of time for the 17 compounds (four to six matrices): 95% LSD intervals for factor means.

determine the method precision and accuracy were carried out at 115 °C.

Figure 5 shows the average recoveries (for data generated for four to six matrices per compound and three temperatures, 80, 115, and 145 °C) and the 95% confidence intervals as a function of time. The average recovery increased slightly with the extraction time, but the increase was not statistically

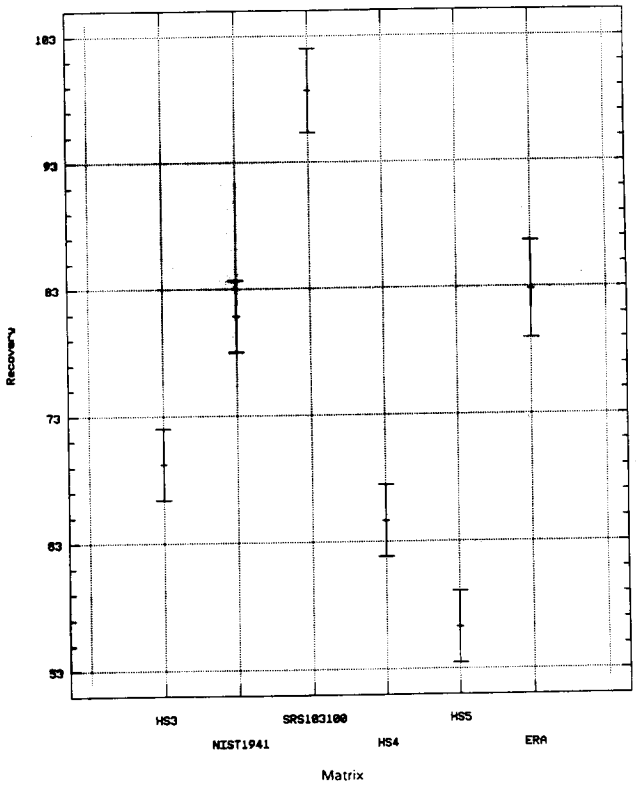

Figure 6. Recovery as a function of matrix for the 17 compounds (four to six matrices): 95% LSD intervals for factor means.

significant. Thus, a 5-min extraction (at 115 °C) was deemed sufficient for recovering PAHs from a soil or sediment matrix.

Figure 6 shows the average recoveries (for data generated for the 17 compounds at three temperatures and three extraction times) and the 95% confidence intervals as a function of matrix. These data indicate that, just as with other extraction methods, method performance was a function of the matrix. It is difficult to establish whether the recovery was also a function of analyte concentration; the results in Figure 6 did not support this. For example, the HS-4 marine sediment matrix had the lowest PAH concentrations (ranging from 0.15 to 1.25 mg/kg), yet the average recovery across the 17 compounds was higher than for the HS-5 marine sediment where the PAH concentrations ranged from 0.2 to 8.4 mg/kg. The NIST sediment material, which had some PAHs at levels that were comparable to those in the HS-4 marine sediment, gave even higher recoveries. The ERA soil, which is not a naturally occurring soil material, but a soil that has been spiked, homogenized, and weathered, exhibited recoveries averaging 83%, which were almost comparable to those measured for the spikes (Figure 7).

The average recoveries (for data generated from four to six matrices per compound at three temperatures and three extraction times) and the 95% confidence intervals as a function of compound are given elsewhere.[9] Of the native compounds, 11 gave average recoveries within 65–85%, and three compounds (acenaphthene, benzo[*a*]pyrene, and fluorene) gave recoveries of ~50%. The average recoveries of the spikes were 77% (compound 4), 105% (compound 6), and 85% (compound 11).

(9) Lopez-Avila, V.; Young, R. EPA Report 600/X-93/XXX, Environmental Monitoring Systems Laboratory, Las Vegas, NV, December 1993.

Table 3. Average Recoveries and Percent RSDs for the Wet and Dry SRS103-100 Soil

compound name	certified value (mg/kg)	MAE[a] dry matrix % recovery (% RSD)[b]		MAE[a] wet matrix % recovery (% RSD)[b]		% RSD extract injection[b]	% RSD std injection[b]
acenaphthene	591	112	(4.6)	111	(6.9)	0.6	1.1
acenaphthylene	16.3	80.9	(5.9)	79.0	(6.0)	0.9	0.8
anthracene	425	107	(4.5)	105	(7.6)	1.4	0.3
benz[*a*]anthracene	249	87.9	(6.2)	84.2	(6.4)	1.5	1.0
benzo[*a*]pyrene	97.5	93.3	(4.6)	87.3	(7.0)	2.2	0.6
benzo[*b*+*k*]fluoranthenes	156	114	(5.0)	111	(7.6)	1.4	0.4
chrysene	310	95.5	(6.3)	92.8	(7.6)	1.8	1.2
fluorene	475	97.3	(4.4)	96.3	(6.3)	1.8	1.3
fluoranthene	1307	109	(4.8)	106	(8.0)	0.7	0.5
2-methylnaphthalene	56.7	98.0	(6.4)	95.0	(6.8)	0.5	NA[c]
naphthalene	23.6	150	(11.7)	143	(13.5)	0.3	0.5
phenanthrene	1450	137	(4.5)	135	(7.5)	0.3	0.3
pyrene	961	117	(4.0)	115	(7.5)	0.7	1.0
dibenzofuran	306	109	(4.6)	108	(6.5)	0.6	1.0
pentachlorophenol	884	80.3	(14.3)	104	(11.8)	11.9	1.1

[a] Six vessels were extracted simultaneously for 10 min at 115 °C. The wet matrix contained 20% water. [b] The number of determinations was six. [c] Data not available.

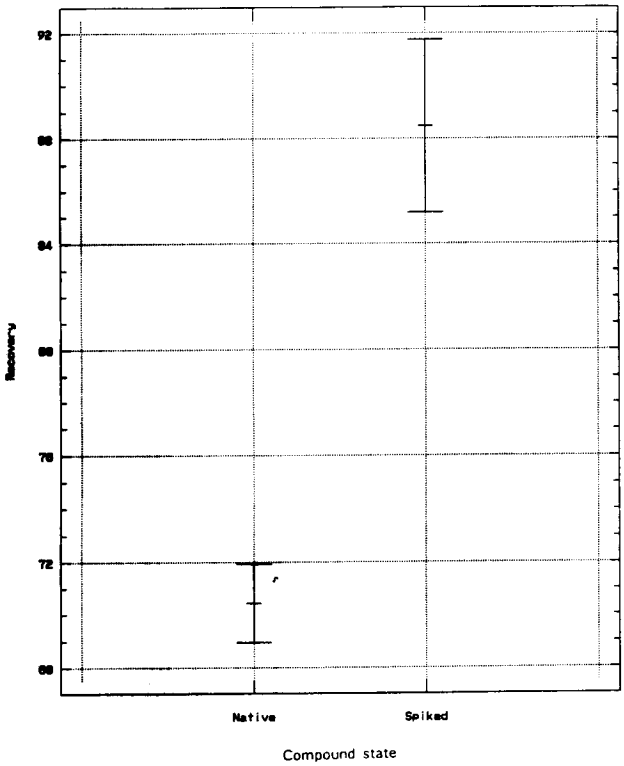

Figure 7. Spiked versus native compound recoveries (four to six matrices): 95% LSD intervals for factor means.

To determine the method accuracy and precision, six 5-g portions of the SRS103-100 standard reference soil were extracted simultaneously for 10 min at 115 °C. The average recoveries and percent RSDs for the 16 compounds known to be present in the sample are presented in Table 3. The repeatability of the injection technique was better than 1.3% (as determined from six consecutive injections of a calibration standard at a concentration of 50 ng/μL). When one of the soil extracts was injected six times, the percent RSDs were comparable to those obtained for the calibration standard, with the exception of pentachlorophenol (% RSD 11.9). The percent RSDs calculated for the six extracts were higher, as expected; but they were under 10% for 14 of the 16 compounds. Naphthalene and pentachlorophenol were the two compounds for which we obtained percent RSDs in the 12–14% range. Percent recoveries established relative to the certified values were greater than 80% and did not appear to be affected by the presence of moisture in the sample. The high recoveries of naphthalene and phenanthrene that we report for the MAE may be due to a certified value that is biased low, since in both cases the standard deviation for the certified concentration is relatively high.

To verify that a 10-min extraction using microwave energy was sufficient to extract the compounds of interest from a soil or sediment matrix, we reextracted the material (remaining after the first extraction) with fresh solvent using the same conditions. Since we did not recover any compounds in the second extraction, we reextracted a separate portion of the material (that had already been extracted once using microwave energy) with methylene chloride/acetone (9:1) using sonication extraction. Except for three compounds (phenanthrene, fluoranthene, and pyrene) that represented less than 5% of their original concentration, the extract was clean. Thus, we concluded that a 10-min MAE was sufficient for the type of matrices we investigated.

Extraction of Other Organic Compounds. The recovery data for other compounds of interest to the EPA that were known to be present in the ERA soil (Lot No. 321) are included in Table 4. The recoveries follow the same trend as in the case of PAHs. The recoveries obtained at room temperature were significantly lower than those obtained using microwave energy; the 5-, 10-, and 20-min extraction results were very similar; and there was a significant improvement in recovery when extractions were done at either 115 or 145 °C as compared with the 80 °C extraction. Compounds that gave recoveries above 80% at 115 °C/10 min included naphthalene, dibenzofuran, *N*-nitroso-di-*n*-propylamine, nitrobenzene, 2,4-dichlorophenol, 2,4,6-trichlorophenol, fluoranthene, chrysene, 2,4-dinitrotoluene, carbazole, and di-*n*-butyl phthalate. Pentachlorophenol exhibited a recovery of 67% and bis(2-ethylhexyl) phthalate a recovery of 79%. 1,2,4-Trichlorobenzene recovery was only 56%, and the more volatile compounds such as 1,2-dichlorobenzene and 2-methylphenol gave low recoveries, even when extracted at room temperature. The

Table 4. Percent Recoveries of "Native" Compounds and Spikes from the ERA Standard Reference Soil (Lot No. 321) with Hexane/Acetone (1:1) at Room Temperature and Using MAE at Different Temperatures and Pressures[a]

compound name	certified value (mg/kg)	room temperature			80 °C/22 psi			115 °C/72 psi			145 °C/150 psi		
		5 min	10 min	20 min	5 min	10 min	20 min	5 min	10 min	20 min	5 min	10 min	20 min
native compounds													
anthracene	3.52	44.0	41.6	39.6	49.3	48.9	54.5	64.5	61.3	64.8	69.5	78.9	63.6
benzo[*b*]fluoranthene	2.03	44.6	43.4	47.6	50.0	51.0	62.1	74.7	68.9	73.4	74.8	80.9	68.8
chrysene	4.47	70.7	65.5	61.6	66.0	69.3	83.7	106	87.2	100	89.7	104	84.3
fluoranthene	8.09	65.2	62.7	61.1	70.2	72.3	83.5	102	89.3	96.6	91.1	102	89.4
naphthalene	2.96	58.6	50.5	46.4	53.9	56.8	64.9	71.4	105	113	160	69.3	66.8
pyrene	3.78	48.7	48.5	44.6	55.6	55.6	66.7	81.2	78.8	79.4	78.3	92.1	79.7
dibenzofuran	1.70	67.4	59.9	53.8	71.4	74.1	80.7	91.6	82.2	84.4	83.6	92.7	81.3
1,2-dichlorobenzene	10.0	33.4	30.5	25.5	31.3	33.8	32.4	37.6	32.7	33.4	29.6	32.3	27.6
N-nitroso-di-*n*-propylamine	5.01	93.7	89.2	79.7	78.0	81.9	84.3	98.7	80.7	85.6	85.9	85.7	74.9
nitrobenzene	7.87	90.2	87.9	74.7	79.4	83.7	84.3	104	82.6	88.6	96.3	98.2	83.4
1,2,4-trichlorobenzene	9.36	52.5	49.4	43.7	53.0	54.6	55.7	67.0	55.7	59.4	54.1	61.9	52.5
2,4-dinitrotoluene	4.86	139	123	112	123	122	140	143	142	144	140	159	125
9*H*-carbazole	5.00	79.4	74.8	65.6	87.6	95.3	107	136	113	120	125	142	118
di-*n*-butyl phthalate	3.43	129	121	112	113	117	141	166	125	145	134	146	130
bis(2-ethylhexyl) phthalate	7.62	53.5	51.4	54.9	59.7	62.2	73.2	92.4	79.1	88.6	85.7	89.8	76.7
2-methylphenol	5.46	20.3	19.2	16.7	20.7	21.1	23.2	25.4	17.9	23.8	24.5	28.0	20.6
2,4-dichlorophenol	5.52	65.0	64.0	57.8	78.7	80.9	79.6	112	87.1	93.2	96.4	103	85.7
2,4,6-trichlorophenol	2.82	59.1	58.2	48.6	71.1	70.8	82.4	94.8	90.9	100	109	129	91.1
pentachlorophenol	6.68	36.3	34.1	35.3	47.6	45.7	56.8	52.5	67.5	76.6	76.8	83.6	76.0
spikes													
anthracene-d_{10}	5.00	87.6	86.4	89.2	86.2	82.3	84.4	83.9	87.9	88.4	96.0	93.8	85.7
fluoranthene-d_{10}	5.00	98.8	89.0	105	95.2	91.3	97.1	92.6	98.0	101	104	100	93.1
benz[*a*]anthracene-d_{12}	5.00	99.2	94.5	103	98.2	91.6	97.7	98.2	96.8	96.6	99.6	101	92.2

[a] Single determinations.

Table 5. Average Recoveries and Percent RSDs for the Dry and Wet ERA Soil (Lot No. 323)

compound name	certified value (mg/kg)	typical rec using approved EPA method[b]	MAE[a] dry matrix % rec (% RSD)[c]		MAE[a] wet matrix % rec (% RSD)[d]		% RSD extract anal.[c]	% RSD std anal.[c]
native compounds								
anthracene	6.97	62.6	72.6	(11.0)	72.0	(2.7)	0.5	0.3
benz[*a*]anthracene	5.16	66.1	74.2	(10.3)	81.3	(5.3)	0.3	1.0
benzo[*k*]fluoranthene	2.35	63.4	49.1	(8.6)	52.3	(4.0)	0.6	2.9
chrysene	9.45	64.0	85.2	(10.5)	83.6	(2.8)	0.7	1.2
naphthalene	7.77	58.7	60.3	(13.2)	59.0	(6.1)	0.4	0.5
pyrene	6.32	66.0	74.7	(9.7)	85.9	(4.9)	0.9	1.0
dibenzofuran	6.50	73.7	80.8	(8.8)	86.1	(2.9)	0.5	1.0
1,2-dichlorobenzene	13.0	43.5	39.1	(18.3)	28.8	(9.6)	0.2	0.4
1,3-dichlorobenzene	8.58	30.5	26.2	(15.8)	17.7	(13.5)	0.4	0.3
1,2,4-trichlorobenzene	1.87	57.2	59.2	(13.1)	55.5	(5.7)	0.7	0.3
2,4-dinitrotoluene	4.82	79.7	106	(14.3)	117	(9.2)	0.7	0.5
bis(2-ethylhexyl) phthalate	1.54	89.6	74.7	(10.0)	81.5	(5.8)	0.9	0.4
isophorone	4.75	67.2	76.5	(11.9)	78.1	(2.4)	0.6	0.5
4-chlorophenyl phenyl ether	3.40	80.6	79.4	(9.3)	87.0	(4.3)	1.1	0.3
butylbenzyl phthalate	8.48	73.6	90.0	(11.5)	100	(8.1)	0.9	0.9
phenol	3.94	65.0	72.2	(18.9)	72.7	(3.8)	0.4	0.8
2-methylphenol	3.41	46.6	34.6	(15.4)	36.3	(5.1)	0.6	0.5
3-methylphenol	4.62	64.9	72.2	(13.9)	74.6	(2.9)	0.6	0.9
2,4,6-trichlorophenol	9.36	57.1	89.0	(14.3)	89.7	(21.8)	1.1	1.0
pentachlorophenol	9.63	42.7	64.6	(39.0)	96.8	(9.4)	5.3	1.1
spikes								
anthracene-d_{10}	5.60		83.1	(11.6)	91.4	(2.7)	0.6	2.9
fluoranthene-d_{10}	5.60		82.4	(8.6)	96.3	(5.0)	0.7	0.5
benz[*a*]anthracene-d_{12}	5.60		85.2	(9.1)	102	(8.7)	4.3	1.4

[a] Five to six samples were extracted simultsneously for 10 min at 115 °C. The wet matrices contained 20% water. [b] Reported by ERA. [c] The number of determinations was six. [d] The number of determinations was five.

average recoveries and the percent RSDs were also determined for this matrix using six 5-g portions of the ERA soil (both dry and wet). Unfortunately, we could not use material from Lot No. 321, since it was no longer available from ERA. However, the soil matrix was the same in the new Lot No. 323, and the spike levels were comparable to those in Lot No. 321. Table 5 identifies the 20 compounds known to be present in ERA soil Lot No. 323, and gives their certified values, the typical recoveries achieved using the EPA-approved methodology (Soxhlet or sonication techniques), and the recoveries that we achieved using MAE. In addition, the percent RSDs are shown for six consecutive injections of one of the extracts and of a calibration standard. The repeatability of the injection technique was better than 1.4% (two of the 23 values were at 2.9%) for the calibration standard and better than 1.1% for the ERA extract (two of the 23 values were at 4.3 and 5.3%).

Table 6. Average Recoveries and Percent RSDs for Phenols after MAE: Solvent versus Solvent/Soil Suspension[a]

	solvent only		solvent and soil		
compound name	% av rec	% RSD	% av rec	% RSD	blowdown evap
phenol	80.3	22.9	74.9	7.6	84.5
2-chlorophenol	87.4	14.6	76.7	8.9	87.1
2-methylphenol	85.0	12.5	75.9	7.4	83.0
3-methylphenol	91.1	11.5	79.1	8.2	86.9
2-nitrophenol	100	20.9	66.7	9.2	85.4
2,4-dimethylphenol	86.9	7.0	76.4	9.7	71.3
2,4-dichlorophenol	87.2	9.3	75.2	6.4	84.1
4-chloro-3-methylphenol	89.4	8.0	78.8	4.6	85.2
2,4,6-trichlorophenol	90.4	8.8	76.9	5.5	84.7
2,4-dinitrophenol	106	21.0	9.4	20.2	82.7
4-nitrophenol	86.7	10.8	74.2	6.7	82.6
2,3,4,6-tetrachlorophenol	91.1	8.9	73.9	5.6	84.4
2-methyl-4,6-dinitrophenol	111	18.7	17.1	34.6	85.7
pentachlorophenol	93.4	9.8	55.0	7.3	81.6

[a] The number of determinations was three.

There was more spread in the average recoveries for the ERA soil since the compounds covered a wider range of volatilities. However, the recoveries that we achieved by the MAE were for the most part higher than those achieved by the EPA-approved methodology. When comparing the typical recoveries achieved with the EPA-approved methodology with those achieved with the MAE for the dry sample (Table 5), we found that 11 compounds exhibited an increase in recovery greater than 10% (the range was 10–56%), two compounds exhibited a 6–7% increase, two compounds showed no change in recovery, and five compounds exhibited a decrease in recovery (10–15%). In the case of the wet matrix, the recoveries for the MAE were higher than those for conventional extraction for 14 of the 20 compounds known to be present in the matrix and lower (but not exceeding 15% difference) for six compounds.

To test for possible compound degradation during the extraction, we performed experiments in which we heated solvent (hexane/acetone, 1:1) and solvent/soil suspensions, spiked with the target compounds, at 115 °C for 10 min at 50% power. To account for possible losses during the nitrogen blowdown evaporation, we took an equivalent volume of hexane/acetone (1:1) and spiked it with the target compounds at the same concentration as the samples subjected to microwave-assisted extraction. The spiked solvent was then concentrated to 1 mL for phenols and PAHs and was analyzed as for organochlorine pesticides. The results are presented in Tables 6–8.

For the 14 phenols tested (Table 6), we did not find any degradation when using solvent only (recoveries ranged from 80.3 to 111%). When soil was present, we found slightly lower but acceptable recoveries (>70%) for 10 compounds, 2 compounds had borderline recoveries (2-nitrophenol at 66.7% and pentachlorophenol at 55%), and 2 compounds (2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol) appeared to have degraded since their recoveries were 9.4 and 17.1%, respectively. Catalytic reactions in the presence of the soil may have been the cause of these low recoveries.

In the case of the organochlorine pesticides (Table 7), we did not find any degradation when using solvent only

Table 7. Average Recoveries and Percent RSDs for Organochlorine Pesticides after MAE: Solvent versus Solvent/Soil Suspension[a]

	solvent only		solvent and soil	
compound name	% av rec	% RSD	% av rec	% RSD
α-BHC	104	1.6	82.4	8.4
β-BHC	103	1.5	81.9	9.3
γ-BHC	104	1.7	88.0	8.5
δ-BHC	107	1.5	95.5	9.5
heptachlor	105	1.5	108	12.2
aldrin	107	1.3	92.5	8.4
heptachlor epoxide	107	2.3	100	11.5
γ-chlordane	83.0	2.3	74.0	11.0
endosulfan-I	110	4.0	98.2	11.4
α-chlordane	99.3	0.5	86.9	9.6
dieldrin	108	2.7	125	14.8
4,4′-DDE	107	0.9	93.9	9.6
endrin	109	0.6	123	14.4
endosulfan-II	106	1.4	99.6	8.4
4,4′-DDD	111	2.0	118	15.4
endrin aldehyde	117	1.0	92.6	11.9
endosulfan sulfate	109	1.5	101	11.0
4,4′-DDT	105	1.0	114	22.7
endrin ketone	107	1.2	123	14.9
methoxychlor	115	6.0	169	17.4

[a] The number of determinations was three. The hexane/acetone extracts were not concentrated.

Table 8. Average Recoveries and Percent RSDs for PAHs and Selected Base/Neutral Compounds after MAE: Solvent versus Solvent/Soil Suspension[a]

	solvent and soil		solvent only		
compound name	% av rec	% RSD	% av rec	% RSD	blow-down evap
PAHs					
acenaphthene	72.6	4.0	103	2.2	95.8
acenaphthylene	74.8	4.1	105	1.9	97.9
anthracene	84.8	2.4	109	3.0	103
anthracene-d_{10}	76.6	2.5	95.5	2.6	89.8
benz[*a*]anthracene	79.5	2.1	103	2.7	95.9
benz[*a*]anthracene-d_{12}	82.4	3.5	106	1.9	98.8
benzo[*a*]pyrene	101	10.3	94.7	2.2	88.1
benzo[*b*]fluoranthene	103	11.1	93.6	2.2	85.2
benzo[*k*]fluoranthene	101	11.6	94.5	4.2	90.7
benzo[*ghi*]perylene	100	8.9	91.0	2.3	81.1
chrysene	81.3	1.7	105	2.2	99.0
fluoranthene-d_{10}	82.1	4.1	105	2.3	99.0
fluorene	77.5	3.9	106	2.8	98.1
fluoranthene	85.0	4.1	109	3.3	102
indeno(1,2,3-*cd*)pyrene	109	9.9	102	2.7	93.3
naphthalene	66.3	5.0	93.3	8.0	90.7
phenanthrene	81.9	2.3	106	3.5	99.0
pyrene	78.1	2.0	102	2.3	94.9
base/neutral compounds					
dibenzofuran	75.2	3.7	105	3.2	96.1
1,2-dichlorobenzene	62.1	5.8	85.2	16.7	86.8
1,3-dichlorobenzene	61.6	7.4	87.5	15.3	88.6
1,2,4-trichlorobenzene	65.3	5.4	93.0	6.8	89.8
2,4-dinitrotoluene	96.7	3.1	135	3.3	126
isophorone	67.4	4.9	96.6	1.6	90.9
4-chlorophenyl phenyl ether	77.1	3.8	107	3.2	97.1
butyl benzyl phthalate	84.5	2.0	112	1.8	105

[a] The number of determinations was three.

(recoveries ranged from 83 to 117%). When soil was present, recoveries were still almost quantitative for all compounds but γ-chlordane (recovery 74%). We found some losses for three of the four BHC isomers; however, their recoveries were acceptable (the range was 82–88%).

In the case of the PAHs and a few base/neutral compounds (Table 8), we did not find any degradation when using solvent

only (recoveries ranged from 85.2 to 135%). When soil was present, PAHs exhibited ~15% loss in recoveries and the other compounds exhibited ~30% loss. Considering that losses during the blowdown step could be as high as 15% for some of these compounds and that the measurement error could also be as high as 15%, we concluded that the 30% loss is not unreasonable for these types of compounds. We believe some of the more volatile compounds (e.g., chlorinated benzenes, naphthalene) may be partially lost during the filtration step.

In summary, MAE of stable organic compounds from soil samples seems to be a viable alternative to the conventional techniques employing Soxhlet/Soxtec and sonication extraction. The main advantages of sample preparation using microwave energy are reduced extraction time (typical sample preparation time for this technique is 10 min for extraction and 40 min for extract cooling, centrifugation, and extract concentration) and reduced solvent use (30 mL in the MAE versus 300 mL in the Soxhlet/Soxtec extraction). Up to 12 samples can be extracted simultaneously in a few minutes, resulting in increased sample throughput over the conventional extraction techniques that employ Soxhlet and sonication extraction.

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