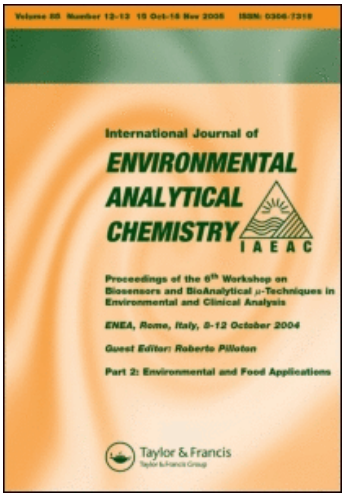

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BIOASSAY AND CHEMICAL ANALYSIS OF AMBIENT AIR PARTICULATE EXTRACTS FRACTIONATED BY USING NONAQUEOUS ANION-EXCHANGE SOLID PHASE EXTRACTION

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A nonaqueous anion-exchange solid-phase extraction technique has been developed for analyzing particulate extracts of ambient air samples and combustion source samples. The technique has been used for sample preparation and sample prefractionation in bioassay-directed fractionations. This technique employs an anion-exchange resin to separate complex particulate extracts into four discrete fractions, which are characterized as neutral/basic, polar neutral/weak acid, weak acid, and stronger acid components. Two ambient air particulate extracts from Boise, Idaho, were analyzed by the developed method. Both samples contain a high percentage of organic compounds associated with wood combustion. The average recoveries of mass and mutagenicity were 93.7± 1.5% and 100.8; pm 2.3%, respectively. Qualitative chemical analysis of the resulting fractions by gas chromatography-mass spectrometry showed agreement with the chemical class fractionation predicted by the separation of standard reference compounds. Polycyclic aromatic hydrocarbons (PAHs), aliphatic carboxylic acids, nitrated phenols and cresols, alkoxy alcohols, and alkoxy phenoxy compounds were some of the compound classes detected; selected PAHs were slightly more abundant in the sample containing higher levels of automotive emissions.

KEY WORDS: Ion-exchange, PAH, organic-acids, ambient-air-particulate-extracts, bioassay-directed-fractionation, nonaqueous solid phase extraction.

INTRODUCTION

The analysis of complex mixtures, such as ambient air particulate extracts or combustion-source particulate extracts, to determine genotoxicity and to identify mutagens has recently been approached by using bioassay-directed fractionation[1–4]. This process requires the fractionation of complex mixtures into discrete chemical classes with near quantitative recoveries of both mass and mutagenicity. In addition, the process requires enhancement of mutagenic potency in selected fractions to direct further chemical and bioassays. Previous bioassay-directed fractionations have met these requirements with varying degrees of success. For example, when diesel particulate extracts were fractionated by using normal-phase high-performance liquid chromatography (HPLC), mass recoveries of 85–100% and mutagenicity recoveries of 70–100% were reported[5–8]. In a study of woodsmoke-source particulate extracts, recoveries of mass ranged between 91 and 98%; however, recoveries of mutagenicity ranged between 50 and 105%[9]. Lewtas[10] observed lower recoveries of both mass and mutagenicity, as well as greater variation, during the normal-phase HPLC fractionation of particulate sample extracts of ambient air that had been highly impacted by woodsmoke.

The difficulties in fractionating woodsmoke particulate extracts are likely due to the presence of polar and acidic compounds that are produced during combustion of wood lignin and its subunits[11–13]. When silica HPLC is used, difficulties with the chemisorption of polar and acidic sample components and with obtaining reproducible chromatographic results are encountered[14–16]. Sample mass recoveries have been improved when organic acids are isolated from complex mixtures prior to silica-HPLC[17]. Although many techniques exist for fractionating organic acids, these methods are principally designed for analyte determinations. Also, techniques that involve buffers and solvent modifiers result in fractions that are incompatible with further chemical and biological analyses[18,19]. One technique compatible with further sample analysis is liquid-liquid extraction, but although this technique was successfully used to fractionate urban air particulate extracts[20,21], it yielded relatively low recoveries of mass when applied to woodsmoke-source particulate matter[22].

Other methods of fractionation that are compatible with further sample analysis include nonaqueous anion-exchange chromatography and solid-phase extraction (SPE). Nonaqueous methods of ion-exchange chromatography have been used to separate petroleum distillates[23–26], and more recently, SPE methods were used to collect vapor-phase phenols[27] and to separate woodsmoke particulate extracts[28]. Although the latter study resulted in acceptable recoveries, the separation of acids from the neutral components was incomplete. In this paper we report a modification of that nonaqueous anion-exchange solid-phase extraction procedure (anion-SPE) that provides complete chemical class fractionations with about 100% recovery of mutagenicity. We report here the evaluation of this technique using reference standards of diverse chemical classes and using ambient air particulate extracts from an air shed impacted by residential woodsmoke emissions. For this evaluation mass and mutagenicity recoveries were determined, and gas chromatography-mass spectrometry (GC-MS) was used to identify characteristic compounds in fractions of the ambient particulate extracts. We also present the application of the anion-exchange technique to pre-fractionate samples for mutagenicity fingerprinting and for elimination of toxic anions.

EXPERIMENTAL

Reagents All solvents—acetonitrile (ACN), water, methanol (MeOH), dichloromethane (DCM), and hexane—were obtained from Burdick and Jackson (Muskegon, MI). The anion-exchange resin, AG MP-1 (100–200 mesh), was obtained from Bio-Rad (Richmond, CA). The trifluoroacetic acid (TFA) and acridine were obtained from Sigma (St. Louis, MO). Propylamine, pyrene, phenanthrene, 7, 8-benzoquinoline, 1-naphthol, 9-phenylanthracene, 4-nitrophenol, and 2-nitrobenzoic acid were obtained from Aldrich (Milwaukee, WI). The acetic acid was obtained from Mallinckrodt (Paris, KY), the CO_2 was obtained from National Specialty Gases (Raleigh, NC), 1-nitropyrene was obtained from Chemsyn Science Laboratories (Lenexa, KS), and the 1-nitronaphthalene was obtained from Chem Service (West Chester, PA). The standard mixtures were prepared in DCM (0.05–1.0 mg/mL), and the acids were dissolved in less than 0.5 mL MeOH prior to dilution with DCM.

Resin preparation The anion-exchange resin, AG MP-1, was prepared for use by converting the resin counter ion from chloride to hydroxide. A 20-mL aliquot of resin (about 20 mequiv, settled in MeOH) was placed in a vacuum filtration unit equipped with a 5.0-μm Teflon filter (Millipore) and sequentially rinsed with the following solvents (adapted from the Bio-Rad protocol and similar to a method reported by Nishioka and Burkholder[27]: 8× (eight rinses) with 25 mL 1.0 N HCl/MeOH (200 mequiv total), 8× with 25 mL MeOH, 8× with 25 mL HPLC-grade water, 8× with 50 mL 1.0 N NaOH/H_2O (400 mequiv), 8× with 25 mL HPLC-grade water, and finally 8× with 25 mL MeOH. After the last rinse of each water wash, the effluent was analyzed for neutral pH. At this stage, the resin could be stored in an amber bottle for 2–3 days. The solid-phase extraction columns were prepared by placing approximately 1.0 mL resin (about 1 mequiv) into a 2.0-mL glass syringe plugged with silanized glass wool. A second plug of glass wool was placed on top of the resin bed to prevent the resin from floating in DCM. A Millipore, Millex-LCR 0.5-μm sample filter (10-mm diameter) was then placed in line between the anion-SPE column and the SPE vacuum manifold. Prior to sample fractionation, the columns were rinsed with 10 mL MeOH and then 10 mL DCM.

Fractionation procedure Ambient air extracts, approximately 50 mg in DCM, were concentrated under nitrogen to approximately 250 μL and placed directly onto the resin. The samples were eluted with 25- mL aliquots of four solvents in the order DCM, MeOH, CO_2/MeOH (prepared by bubbling CO_2 in MeOH for 15 min), and 5% TFA/MeOH. The flow of solvent was maintained by using a constant 20-psi vacuum (about 2.5–5.0 mL/min). The DCM, MeOH, and CO_2/MeOH fractions were concentrated on a rotary-evaporator to approximately 2 mL, quantitatively transferred to a 10-mL volumetric flask, and diluted to volume with DCM. After initial concentration of the TFA/MeOH fraction by rotary evaporation, the residue was diluted to approximately 50 mL with MeOH and then concentrated to approximately 1 mL. This procedure was repeated 5 to 10 times until all the TFA was removed. Finally, the TFA fraction was transferred to a 10-mL volumetric flask and diluted to volume with MeOH.

Gravimetric analysis Gravimetric analyses were performed on a Sartorius ultra-micro balance. Fraction masses were determined in duplicate by placing 500 μL into each of two

aluminum weigh pans. The sample measurements were repeated with greater volume if the aliquot mass was less than 100 μg. Four ambient blanks were measured, averaged, and used to adjust for changing ambient conditions.

Microbial mutagenicity methods The collected fractions were assayed for mutagenic activity toward *Salmonella typhimurium* strain TA98 by using the Ames plate incorporation assay[29] with exogenous metabolic activation (+S9) according to published guidelines[30]. The fractions were tested with duplicate plates at five dose levels of 6.25, 12.5, 25.0, 50.0, and 100 μg. The concentration of mutagenicity is referred to as mutagenic activity or potency and is defined as the number of observed mutagenic events (revertants) in 1 μg of sample (rev/μg). Mutagenic potency was defined as the slope of the dose-response data which was calculated after Bernstein's[31] analysis of the dose-response curves for indications of toxicity.

A microsuspension mutation assay in *S. typhimurium* strain TA98 was used to measure the mutagenic activity of HPLC fractions, as described by Lewtas *et al.*[1]. The bioassay procedure is described in detail by DeMarini *et al.*[32] and is a modification of the microsuspension assay developed by Kado *et al.*[33].

HPLC All HPLC experiments were performed on a Beckman System Gold HPLC with a model 166 programmable UV detector. Recoveries of neutral and basic reference compounds through anion-SPE fractionation were determined by using reversed-phase HPLC with a Du Pont (Wilmington, DE) Zorbax ODS reversed-phase HPLC column (0.46×25 cm; 10-μm particles). A step gradient program was initiated at 42% ACN/water for 4 min, followed by a 1-min step to 65% ACN/water that was held for 4 min, and finally a 1-min step to 85% ACN/water which was held for 4 min; all solvents used were modified with 0.2% propylamine, and the flow rate was 2.0 mL/min. Phenanthrene, 1-nitropyrene, and pyrene were detected at 265 nm, and the remaining compounds were detected at 235 nm.

Recoveries of acidic reference compounds through anion-SPE were determined by using a Keystone Scientific (State College, PA) Deltabond cyanopropyl (CN) HPLC column (0.46×25 cm; 5-μm particles) in the normal-phase mode. The HPLC procedure involved a linear gradient of 5–15% DCM/hexane over 10 min; this was followed by a 2-min step to 50% DCM/hexane, which was then held for 4 min at the final solvent composition. All solvents were modified with 0.6% acetic acid, a flow rate of 2.0 mL/min was used, and detection was at 250 nm.

Further separation of the anion-SPE TFA/MeOH fractions was performed by using the Deltabond cyanopropyl HPLC column in the normal-phase mode. The method involved five steps: 100% hexane for 10 min, 0–100% DCM/hexane over 10 min, 100% DCM for 10 min, 0–100% MeOH/DCM over 10 min, and finally 100% MeOH for 10 min. The flow rate was 2.0 mL/min, and detection was at 250 nm.

Preparation and characterization of particulate extracts The ambient air particulate extracts were collected during the U.S. EPA Integrated Air Cancer Project field study in Boise, Idaho, conducted between November 1986 and February 1987. Boise has a simplified air shed, whose pollutant burden is generated mainly by residential wood combustion and motor vehicles, both of which can contribute substantially to the mutagenic and carcinogenic activity found in the extractable organic matter (EOM) from ambient air aerosols. Descrip-

tions of the site and collection media have been presented in detail elsewhere[34]. Briefly, daytime and nighttime 12-h samples were collected on Teflon-impregnated glass fiber filters by using hi-vol samplers equipped with 0–2.5-μm impactors. Two primary ambient sampling sites were selected in Boise where residential wood combustion or automotive exhaust were expected to dominate. Particulate extracts were composited to produce two samples that would represent these two source categories with enough EOM for chemical and biological studies. Source apportionment receptor modeling techniques were used to select the samples to be included in each composite. First, a chemometric procedure[35] was used to select 25 daytime and 25 nighttime samples from each site (100 total) for independent extraction (Soxhlet-DCM). A single-element-tracer multilinear regression (MLR) model was then applied to the EOM data by using soil-corrected fine particle potassium, K′, and fine particle bromine (X-ray fluorescence data from collocated dichotomous samplers) as tracers for emissions from wood combustion and mobile sources, respectively[36]. With the coefficients determined by MLR, the expected contributions of woodsmoke and automotive sources to the EOM for all samples were calculated and the samples ranked by source contribution. Two large-scale extractions were then conducted on composites of additional filters grouped according to the above ranking to maximize the two sources. The woodsmoke composite sample (WSC) was 78.3% woodsmoke, 11.3% mobile source, and 10.4% residual mass. The mobile source composite sample (WS-MSC) was 51.4% woodsmoke, 33.4% mobile source, and 15.2% residual mass.

GC-MS analysis methods The electron ionization (EI) and negative chemical ionization (NCI) GC-MS analyses were carried out by using Finnigan 4500 mass spectrometers. For the EI analyses, heated splitless injection was used, and the DB-5 column was programmed from 50 to 300°C at 6°C/min. Helium was used as the GC carrier gas. The ionizing energy was 70 eV. For the NCI analyses, cool on-column injection was used, and the DB-5 column was programmed from 40 to 100°C at 20°C/min and then from 100 to 300°C at 8°C/min. Helium was used as the GC carrier gas; methane was used as the reagent gas at a nominal source pressure of 1 torr and ionizing energy of 150 eV for production of the thermal electrons. Spectra were acquired every 1 s from 40 to 450 daltons. For EI GC-MS analyses of the DCM fractions, the organic mass concentrations were adjusted to equal the proportion that the DCM fraction was to the total sample mass. An internal standard, 9-phenylanthracene, was added to both fractions at the same concentration just prior to analysis.

RESULTS AND DISCUSSION

Chemical class fractionation of reference compounds Recovery and distribution of nine representative compounds through the anion-SPE method are given in Table 1. These data indicate that the neutral and basic compounds, pyrene, phenanthrene, 7,8-benzoquinoline, 1-nitropyrene, 1-nitronaphthalene, and acridine, eluted almost entirely in the DCM fraction and that less than 3% eluted in the MeOH fraction; the average mass recovery of each standard was about 91%. The weak acid, 1-naphthol, eluted in both the MeOH and CO_2/MeOH fractions for a combined recovery of 90%. The elution of this analyte in both

Table 1 Recoveries of reference compounds in the four solvents,%*.

Analyte	*DCM*	*MeOH*	*CO_2/MeOH*	*TFA/MeOH*	*Total*	*n*
NEUTRAL						
pyrene	89.9 (2.5)	2.9 (3.8)	-	-	92.1 (4.5)	8
phenanthrene	88.3 (2.5)	-	-	-	88.3 (2.5)	2
7,8-benzoquinoline	92.2 (0.5)	0.5 (0.2)	-	-	92.7 (0.8)	2
1-nitropyrene	91.3 (3.6)	-	-	-	91.3 (3.6)	6
1-nitronaphthalene	91.8 (0.3)	0.5 (0.2)	-	-	92.3 (0.5)	2
BASE						
acridine	89.9 (2.6)	-	-	-	89.9 (2.6)	6
ACID						
1-naphthol	-	50.8 (7.2)	38.8 (6.1)	-	89.7 (2.1)	4
4-nitrophenol	-	-	-	105.0 (4.0)	105.0 (4.0)	2
2-nitrobenzoic acid	-	-	-	96.3 (10.3)	96.3 (10.3)	4

*Standard deviations are given in parentheses.

the methanol and acidic methanol fractions suggests weak adsorption of 1-naphthol to the solid phase. Finally, the stronger acids, 4-nitrophenol and 2-nitrobenzoic acid, eluted entirely in the TFA/MeOH fraction with recoveries greater than 95%. Although under the conditions used in this experiment 4-nitrophenol eluted entirely in the TFA/MeOH fraction, partial elution of this acid in the CO_2/MeOH fraction was possible when 1–5 mL additional CO_2/MeOH was used (data not shown).

Chemical class fractionation of ambient air particulate extracts The anion-SPE method was used to fractionate 50-mg replicate samples of both the WS-MSC and the WSC particulate extracts. The recoveries of mass and mutagenicity (TA98 +S9) for the extract fractions are given in Table 2. As suggested by the mass recovery data, the fractionation procedure was quantitative and repeatable. For both extracts, the mass recovery was greater than 90%, and the fraction mass deviation from the mean was generally less than 5%. These data also suggest that most of the mass in these samples is associated with the polar and acidic fractions (MeOH, CO_2/MeOH, and TFA/MeOH); the sum of the mass recovered in these fractions is 59% for the WS-MSC sample and 76% for the WSC sample. Mass recoveries in blank samples were 0.349 mg in the DCM fraction, 0.509 mg in the MeOH fraction, 0.556 mg in the CO_2/MeOH fraction, and 2.122 mg in the TFA/MeOH fraction.

The mutagenicity data demonstrated again that the fractionation procedure was quantitative and repeatable. The recovery of mutagenicity in both samples was about 100% with

Table 2 Recovery of mutagenic activity and mass for replicate samples.

Fraction	*Mass Recovery, %*	*Mutagenic Activity Recovery, %*	*Mutagenic Activity, rev/μg*
WSC-MSC			
DCM	31.7 ± 0.3	60 ± 2	2.13 ± 0.08
MeOH	26.7 ± 0.9	12 ± 2	0.48 ± 0.08
CO_2/MeOH	17.2 ± 1.5	14 ± 2	0.90 ± 0.03
TFA/MeOH	15.0 ± 1.0	16 ± 1	1.20 ± 0.17
Total	90.6 ± 0.2	101 ± 3	
	Unfractionated Activity	1.13 ± 0.10 rev/μg	
	Reconstituted Activity	1.08 ± 0.03 rev/μg	
	Mass-Weighted Activity	1.11 ± 0.03 rev/μg	
WSC			
DCM	22.6 ± 0.2	49 ± 9	2.06 ± 0.36
MeOH	31.2 ± 2.4	15 ± 4	0.46 ± 0.15
CO_2/MeOH	23.1 ± 2.5	28 ± 4	1.24 ± 0.21
TFA/MeOH	21.6 ± 6.5	10 ± 3	0.44 ± <0.01
Total	98.4 ± 1.5	101 ± 2	
	Unfractionated Activity	0.95 ± 0.20 rev/μg	
	Reconstituted Activity	0.92 ± 0.07 rev/μg	
	Mass-Weighted Activity	0.97 ± 0.02 rev/μg	

less than a 10% relative deviation from the mean. These data also suggest that the anion-SPE method concentrated approximately half of the mutagenicity into the DCM fraction, which contained only 20–30% of the sample mass. The mutagenic activity of each of the fractions is also shown in Table 2. These data support our observation that the most potent fraction of both samples is the DCM fraction.

The unfractionated, reconstituted, and mass-weighted mutagenic activities of both samples are also listed in Table 2. The reconstituted activities for both samples are comparable with each of the unfractionated activities. This suggests that the fractionation procedure has little effect on sample mutagenicity. The mass-weighted mutagenic activity is calculated by multiplying the fraction's mutagenic activity by the recovered mass and then adding the results from each fraction [$\sigma_{i=}$DCM. .TFA(fraction(i) mass recovery * fraction(i) potency)]. These values are again comparable with the unfractionated mutagenic potency. This correlation suggests that the mutagens, even when split into four fractions, are not affected by the fractionation procedure. (Noncorrelation would provide evidence of synergistic or antagonistic effects on mutagenicity within the complex mixture.) No mutagenicity was observed in method blank fractions.

GC-MS chemical characterization The major compound class fractions that were identified from the EI GC-MS analyses are listed in Table 3. These identifications, in general,

Table 3 Major compound classes identified in WS-MSC and WSC fractions.

Fraction	*Compound Class*	
DCM	alkane	aliphatic aldehyde
	PAH/alkyl PAH	aromatic ketone
	phthalate ester	aliphatic amide
	alkoxy alcohol	alkoxy phenoxy compound
MeOH	phthalate ester	aliphatic carboxylic acid
	alkoxy alcohol	
CO_2/MeOH	alkoxy alcohol	aliphatic carboxylic acid
	alkoxy phenoxy compound	hydroxylated aromatic
TFA/MeOH	alkoxy alcohol	aliphatic carboxylic acid
	methyl ester of aliphatic – carboxylic acid	methyl ester of aromatic – carboxylic acid

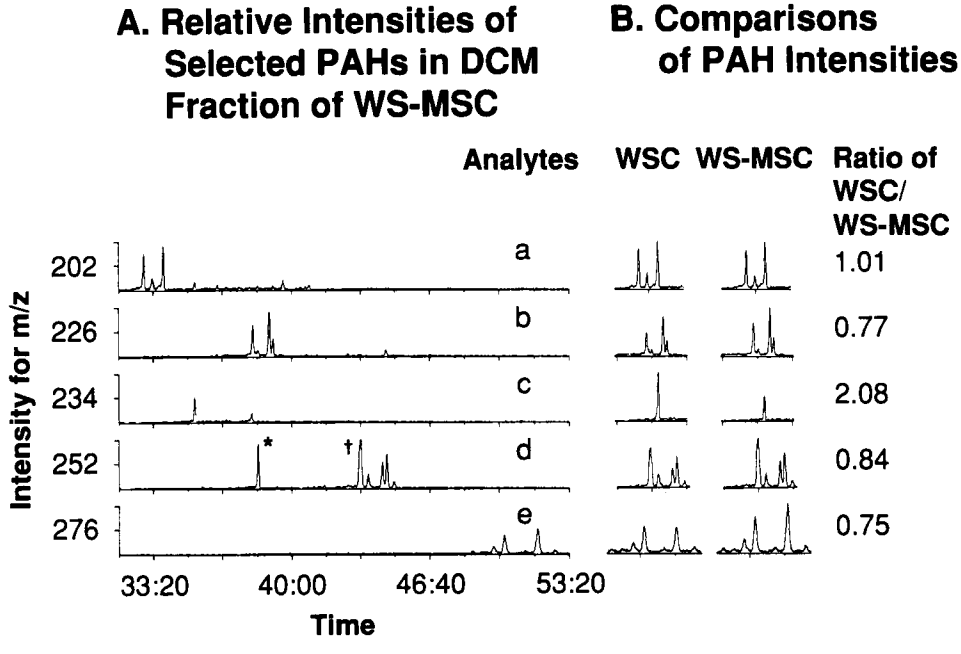

Figure 1 Ion current chromatograms for molecular ions of selected PAHs. (A) Comparison of relative abundance of molecular weight 202-, 226-, 234-, 252-, and 276-dalton PAHs in the WS-MSC DCM fraction. a) fluoranthene, pyrene; b) benzo (gh) fluoranthene, cyclopenta (cd) pyrene; c) retene; d) benzofluoranthenes, b(e)p, b(a)p, perylene; e) indeno (1,2,3-cd) pyrene, benzo (ghi) perylene. (*) indicates M-2 ion at *m/z* 252 of the internal standard; not a native PAH. (†) Indicates most intense peak at 43.2 min identified as benzofluoranthenes. (B) Comparison of relative abundances of selected PAHs in the WSC and WS-MSC DCM fractions. Ratio is based on the summed molecular ion area of all isomers. M-2 ions from molecular weight 228-, 254-, and 278-dalton PAHs, which elute in the window of the 226-, 252-, and 276-dalton PAHs, are not included in the summed area.

agree with the reference compound class fractionation shown in Table 1. In the DCM fractions, the polycyclic aromatic hydrocarbons (PAHs) are not the most abundant compound class identified, but are a major source of mutagenicity. Figure 1 shows the relative levels of several PAHs in the two DCM fractions. Ion current chromatograms are shown in Figure 1A for PAHs of molecular weights 202, 226, 234, 252, and 276 daltons in the WS-MSC DCM fraction. Ion intensities are normalized to the abundance of the most intense peak (benzofluoranthenes; 252 daltons; scan 43.2 min). As seen there, the PAHs of 202 and 226 daltons were nearly as abundant as these benzofluoranthenes.

As discussed in the experimental section, the organic mass concentrations of the DCM fractions were adjusted prior to GC-MS analysis to reflect the proportion that the DCM fraction is to the total extract. This output then can be used to compare visually the relative levels of these PAHs in the two samples. The molecular ion peak area, summed for all isomers, was used to compute the PAH ratio of WSC to WS-MSC. These ratios suggest that the sum of the fluoranthene, acetophenanthrylene, and pyrene concentrations is nearly equivalent in the two samples (ratio of 1.01), that retene is twice as abundant in the WSC sample (ratio 2.08), and that the PAHs of 226, 252, and 276 daltons are more abundant in the WS-MSC sample by 20–30% (ratios of 0.77, 0.84, and 0.75, respectively).

Several aliphatic amides were present in both DCM fractions. One, tentatively identified as $C_{22}H_{43}NO$ (molecular weight 337), was a major component of the WSC sample. These were not present in the method blanks.

For the MeOH fractions, in addition to the three major classes of compounds listed in Table 3, several multifunctional oxygenated aromatic and cyclic compounds were tentatively identified. The mass spectrum of one of these compounds and the EPA/NIST reference mass spectrum of 1-phenyl-1-hexen-3-one semicarbazone are shown in Figure 2. The similarity of the two spectra suggests that the species identified in the WSC-MeOH fraction is related to this amide-type compound. The aliphatic carboxylic acids in the methanol fractions appear to be isomers of octanoic acid. Because the pKa of octanoic acid is approximately 5, we expected these compounds to elute from the resin in an acidic fraction. Two hypotheses to explain their presence in this polar neutral fraction are hydrophilic interaction with the resin and/or the slight acidity of the methanol due to carbonates formed from dissolved CO_2 and water in the methanol.

Higher molecular weight aliphatic carboxylic acids (C_{12}-C_{18}) were detected in the CO_2/MeOH and TFA/MeOH fractions, together with alkoxy alcohols and alkoxy phenoxy compounds. Some hydroxylated aromatics tentatively identified include hydroxylated methoxybenzaldehyde isomers, hydroxylated methoxy benzoic acid isomers, and hydroxylated fluorene carboxaldehyde. One tentatively identified compound of the CO_2/MeOH fractions, 6-amino-hexanoic acid, appears to be present in greater levels in the WS-MSC extract than in the WSC extract.

The TFA/MeOH fractions contain a greater number of unknown mass spectra. The most abundant class appears to be the aliphatic carboxylic acids. We assume that the neutral species identified in these acidic fractions are compounds which are retained strongly by the resin backbone and whose elution is governed more by the solvent volume than by the pH of the elution solvent.

The NCI GC-MS analysis of the WS-MSC DCM fraction revealed very few major or minor components other than those already detected in the EI GC-MS analysis. The primary

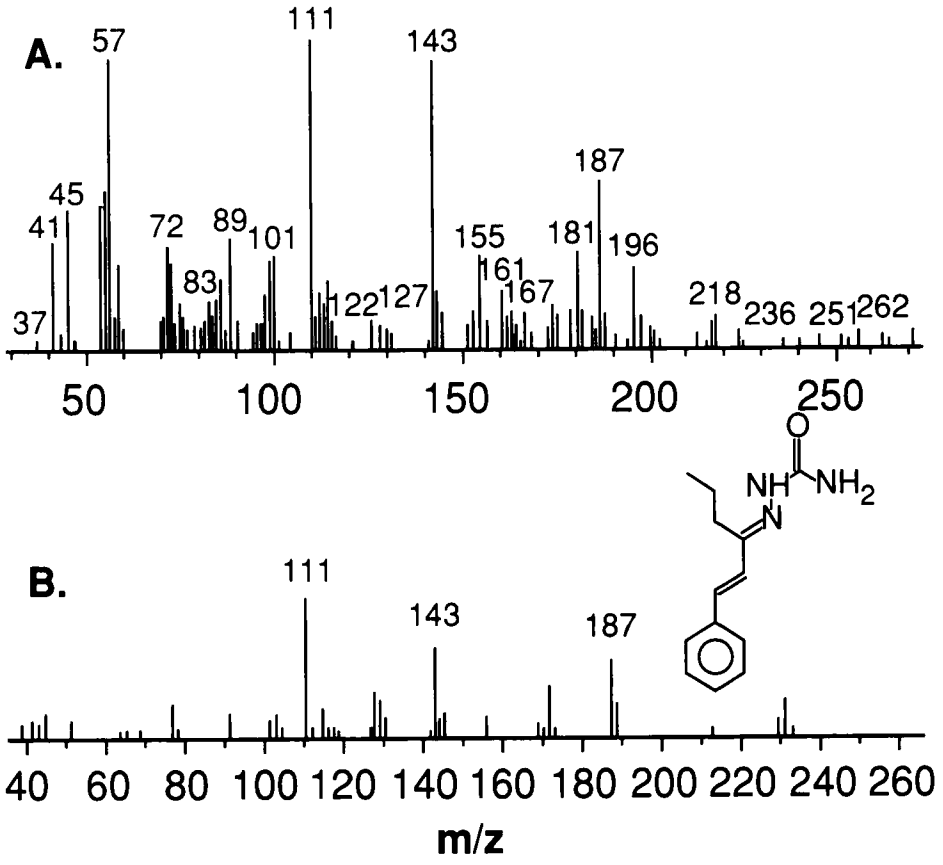

Figure 2 EI mass spectra of (A) component of WSC and WS-MSC MeOH fractions and (B) reference spectrum of indicated 1-hexene-3-one, 1-phenyl-, semicarbazone, suggesting similarity between the two species.

difference between the two analyses was that aliphatic compounds (alkanes, alkoxy alcohols, aliphatic aldehydes, etc.) were not detected in the NCI mode. The major species detected were the PAHs of molecular weight 226, 252, and 276 daltons.

The NCI GC-MS analysis of the WSC CO_2/MeOH fraction showed relatively few components. The species detected included a NO_2-phenol (identified by fragmentation as 4-NO_2-phenol), two methyl-NO_2-phenols (identified by spectrum and by retention relative to 4-NO_2-phenol as 3-methyl-4-NO_2-phenol and 2-methyl-4-NO_2-phenol), several dimethyl-NO_2-phenols, a dichlorophenol, and several tentatively identified hydroxylated polycyclic compounds (including hydroxylated acridine and hydroxylated methylacridine). The presence of the NO_2-phenols suggests that other hydroxylated-NO_2 aromatic and polycyclic compounds may be present at lower levels and may contribute to the mutagenicity of this fraction.

The total ion current chromatograms from the EI and the NCI GC-MS analyses of the WS-MSC DCM fraction and WSC CO_2/MeOH fraction are shown in Figure 3. Major compounds and compound classes are indicated therein. These fractions were analyzed by

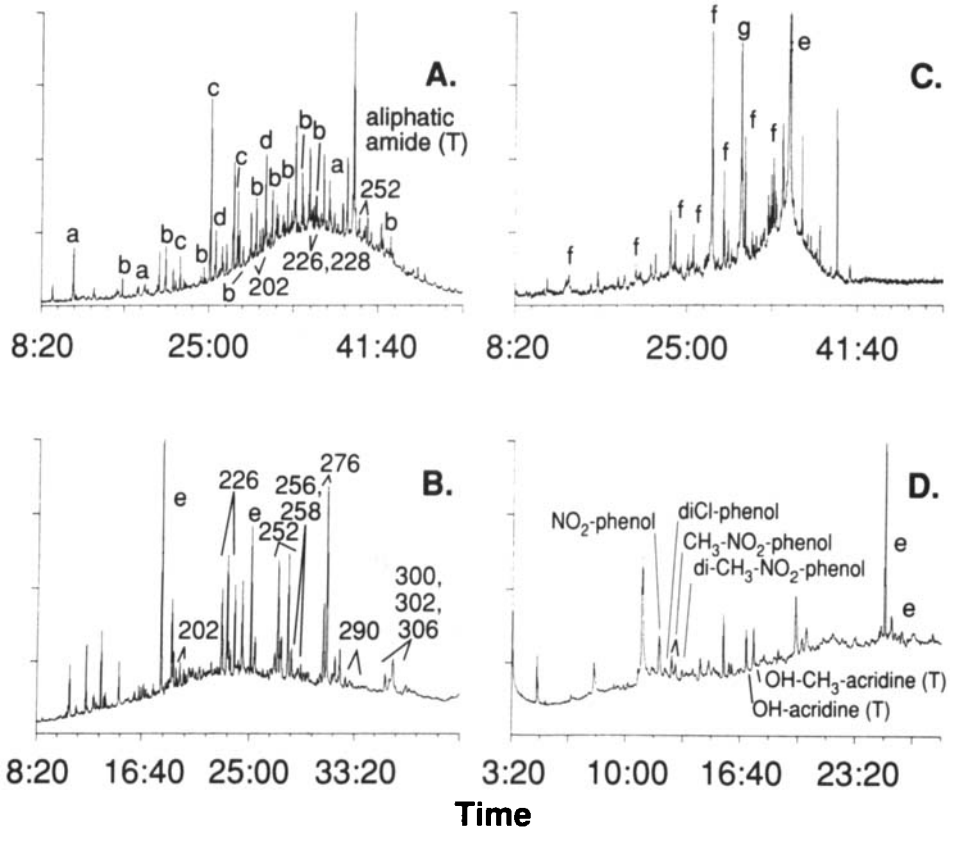

Figure 3 EI and NCI GC-MS chromatograms of selected fractions. (A) EI GC-MS of WS-MSC DCM fraction, (B) NCI GC-MS of WS-MSC DCM fraction, (C) EI GC-MS of WSC CO_2/MeOH fraction, and (D) NCI GC-MS of WSC CO_2/MeOH fraction. a) alkoxy alcohol, b) alkane, c) siloxane, d) alkoxy phenoxy, e) phthalate ester, f) aliphatic carboxylic acid, g) methyl ester of aliphatic carboxylic acid; numbers represent PAH molecular weight. Note: EI GC-MS chromatogram of WS-MSC (A) results from a different analysis than the one presented in Figure 1.

NCI GC-MS to determine whether major differences existed between the most abundant species (as determined by EI GC-MS) and the most electronegative species (as determined by NCI GC-MS) of a fraction. The use of NCI GC-MS has been suggested by Nishioka *et al.*[2] as an approach for selecting potential mutagens in a complex mixture. High electronegativity, whether measured in the gas phase or in solution phase, shows some empirical correlations with mutagenicity[37,38].

The comparisons of the chromatograms from the two NCI analyses and the mutagenicity data for these two fractions tend to confirm our empirical observations about NCI GC-MS as a mutagen sensor. Both DCM fractions were approximately twice as mutagenic as the CO_2/MeOH fractions, and for similar mass quantities analyzed by NCI GC-MS, both the number of analytes detected in NCI and their NCI responses were higher in the DCM fraction relative to the CO_2/MeOH.

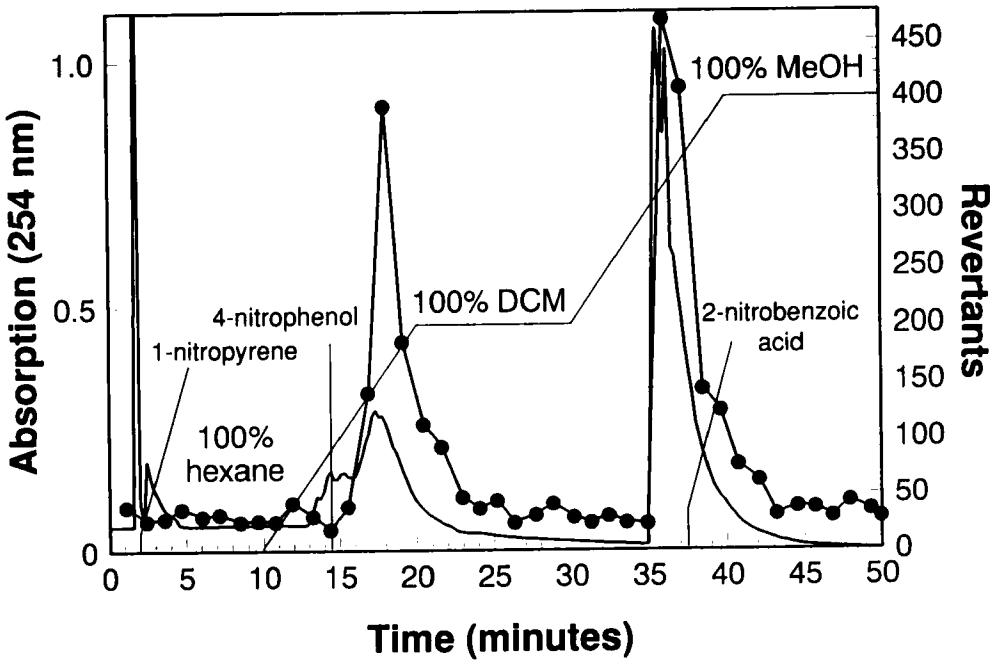

Figure 4 Mutagram of WS-MSC acid fraction separated on a CN-HPLC column in the normal-phase mode. The chromatogram, 254 nm (solid line), sample mutagram (solid line with points) are shown with the gradient overlaid.

APPLICATIONS

Further fractionation for mutagenic profiling As the first level of fractionation, anion-SPE permits the comparison of mutagenicity exhibited in each of the four general chemical classes. Further separation of each fraction can yield a more detailed "fingerprint" of the sample mutagenicity[39,40]. For example, Figure 4 displays a mutagenicity fingerprint (mutagram) of the WSC strong acid fraction (TFA/MeOH) separated on a cyanopropyl HPLC column in the normal-phase mode. The UV chromatogram, elution time of selected chemical markers, and sample fraction bioassay results are shown in Figure 4. The mutagenicity is principally associated with compounds eluting with retention times slightly longer than the 4-nitrophenol standard and compounds eluting with retention times similar to the 2-nitrobenzoic acid standard.

The advantage of using anion-SPE as a chemical class prefractionation step is threefold. First, the anion-SPE method eliminates the need for aqueous liquid-liquid partitioning with associated losses and/or poor recoveries of highly polar compounds. Second, the isolation of the acidic components from the neutral/basic components enables us to choose the appropriate fractionation technique—bonded silica for the acidic fractions, and silica for the neutral/basic fractions. A cation-exchange prefractionation of the neutral/basic fraction may be necessary if mass recoveries are low because of the chemisorption of sample bases onto the silica. Third, the anion-SPE method permits chemical class characterization of observed sample mutagenicity.

Unmask Mutagenicity The anion-SPE technique can also be used as a prefractionation step to remove toxic anions and acids. The toxicity of some species in complex mixtures is so great that the sample mutagenicity cannot be determined. If the toxic materials can be removed, the sample mutagenicity is "unmasked" and can be quantified. One class of samples with a highly toxic mask is particulate extracts of municipal incinerator stack samples[41]. These samples can be extremely acidic, as evidenced by the creation of holes and pitting in aluminum weigh pans during gravimetric analysis. These acidic samples are often toxic to bacteria, which generates negative mutagenicity bioassay results. Anion-SPE fractionation of one such sample resulted in the unmasking of highly mutagenic fractions, and a total reconstituted potency of about 17 rev/μg. The total mass recovery of this sample was only 33%, suggesting the presence of significant quantities of toxic anions or strong acids, which adsorb more strongly to the anion-exchange resin than to the TFA (pKa <1).

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Table 4 Abbreviations.

Abbreviation	*Description*
ACN	acetonitrile
anion-SPE	anion-exchange solid phase extraction
DCM	dichloromethane
EOM	extractable organic matter
EI	electron ionization
GC-MS	gas chromatography-mass spectroscopy
HPLC	high-performance liquid chromatography
MeOH	methanol
NCI	negative chemical ionization
ODS	octadecylsilane
PAHs	polycyclic aromatic hydrocarbons
TFA	trifluoroacetic acid
WSC	woodsmoke composite sample
WS-MSC	mobile source composite sample (51.4% woodsmoke)

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