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Human Cell Mutagens in Respirable Airborne Particles from the Northeastern United States. 2. Quantification of Mutagens and Other Organic Compounds

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Few reports have characterized mutagenic compounds in respirable airborne particles (<2.5 micrometers in diameter; $PM_{2.5}$) collected at different sites on a regional scale (hundreds of km). Previously, we reported differences in the human (h1A1v2) cell mutagenicity of whole and fractionated organic extracts of $PM_{2.5}$ samples collected in Boston, MA, Rochester, NY, and Quabbin Reservoir, a rural site in western MA. Herein we describe the analysis of mutagens and other organic compounds in these samples. Gas chromatography−mass spectrometry (GC−MS) was used to quantify ∼150 organic compounds, including 31 known human cell mutagens. Molecular weight (MW) 226−302 amu PAHs were the most important mutagens identified: cyclopenta[*cd*]pyrene accounted for 1−2% of the measured mutagenicity of the samples, MW 252 PAHs accounted for 4−6%, MW 276−278 PAHs accounted for 2−5%, and MW 302 PAHs accounted for 2−3%. 6*H*-benzo-[*cd*]pyren-6-one, a PAH ketone, accounted for 3−5% of the mutagenicity. The same compounds accounted for similar portions of the total attributed mutagenicity in each sample. Mutagen levels were similar in the Boston and Rochester samples, and both were significantly higher than the Quabbin sample. This may explain why the mutagenicities of the Boston and Rochester samples were higher than the Quabbin sample. The levels of mutagens found in semipolar fractions, however, could not explain why the mutagenicity of semipolar fractions was 2-fold higher in the Rochester sample than in the Boston sample. Known mutagens accounted for only 16−26% of the total mutagenicity of the unfractionated extracts, and only ∼20% of the mutagenicity of the nonpolar and semipolar fractions. The remaining mutagenicity is likely attributable to other, as-yet unknown, semipolar and polar mutagens, or to interactions among chemical constituents of the samples. These findings are consistent with similar studies performed on airborne particles from Los Angeles and Washington, DC, thus indicating that PAHs, PAH-ketones, and as-yet unidentified polar organic compounds are widely distributed airborne human cell mutagens.

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Introduction

Human exposure to respirable airborne particles (<2.5 μm in aerodynamic diameter; $PM_{2.5}$) is associated with increased risks of cardiopulmonary and lung cancer mortality (*1*). Although the mechanisms by which $PM_{2.5}$ causes adverse health effects have not been established, $PM_{2.5}$ typically contains hundreds to thousands of organic and inorganic compounds, many of which are toxic (*2*); therefore, it has been hypothesized that chemicals in the particles may play an important role. To determine which chemicals in airborne particles are most biologically active, a technique referred to as bioassay-directed chemical analysis has been developed. In this method whole particle extracts are separated into fractions, which are tested for biological activity, and the most active fractions are then subjected to detailed chemical analyses (*3*).

In many studies involving bioassay-directed chemical analysis of airborne particles, assays based on the bacterium *Salmonella typhimurium* have been used to assess mutagenicity, the ability to cause inheritable changes in DNA (e.g., *4*−*7*). This combination of bioassay-directed chemical analysis and *S. typhimurium* mutagenicity assays has been used to identify many mutagens in airborne particles. The most widely reported are polycyclic aromatic compounds (PACs) including polycyclic aromatic hydrocarbons (PAHs), nitro-PAH, nitro-PAH lactones, and oxy-PAH (*8*−*12*), many of which are carcinogenic (*13*). Little work has been done to combine bioassay-directed chemical analysis with human cell mutagenicity assays (*14, 15*), in part because until recently metabolically competent human cells were not available for routine mutagenicity testing.

Previously we collected samples of $PM_{2.5}$ every 6th day for 1 year at three sites in Massachusetts (MA; two urban and one rural) and two sites in upstate New York (NY; one urban and one rural) (Figure 1). We then tested bimonthly composites of the samples (n = 7−9 one-day samples per composite) for mutagenicity in a human cell bioassay to see if there were spatial and temporal differences in mutagenicity (*16*). In addition, we created annual composite samples by

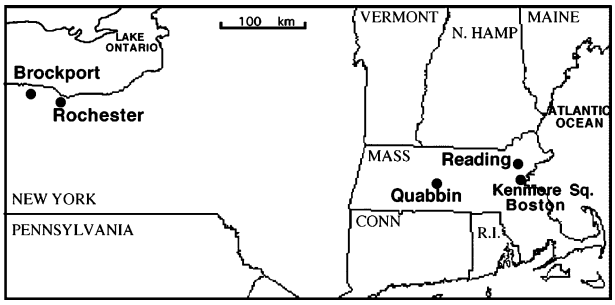

FIGURE 1. Map of the northeastern United States and the 5 sampling sites. Analysis of Rochester, Quabbin, and Kenmore Square (Boston) samples are described herein.

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combining portions of all the samples collected at each of the five sites ($n = 50-54$ samples per site), and tested whole extracts and fractions of the annual composites for mutagenicity (*17*). Our results show that (i) mutagenic potency levels (induced mutant fraction per mass of organic carbon) were significantly higher in winter than in summer at all five sites, (ii) mutagenicity per m^3 of air increased 1.5−2-fold from rural to urban areas within each state, (iii) the mutagenic potency of semipolar fractions of annual composite samples from the five sites was higher than that of nonpolar and polar fractions, and (iv) the mutagenic potency of semipolar fractions at the NY sites was ∼2-fold higher than at the MA sites. We also found that the manner in which samples were composited influenced the mutagenic potency results: mutagenic potency of the annual average of the bimonthly composites in NY were ∼2-fold higher than in MA; however, the mutagenic potency of the annual composite samples from the five sites were not significantly different at the 95% confidence level.

In this paper we test the hypothesis that mutagenic compounds, either known human cell mutagens or other compounds that have not been tested for mutagenicity in h1A1v2 cells, can explain the mutagenicity measured in the particles, as well as spatial differences in particle mutagenicity. Our objectives were to (1) quantify PACs, especially those that are known human cell mutagens, in particle extracts and their fractions, (2) identify compounds that contribute significantly to the observed mutagenicity, and (3) determine whether spatial differences in mutagen concentrations are consistent with spatial differences in the human cell mutagenicity results. The contribution of individual mutagens to the total mutagenicity of the samples was calculated using a simple linear additivity model, which would not account for possible antagonism or enhancement of mutagenicity due to interactions among mutagens and other sample constituents. To date only one other study has reported on the chemical composition of human cell mutagens in $PM_{2.5}$ collected at multiple sites in an urban area (*14*). The present study expands our knowledge of human cell mutagens present in $PM_{2.5}$, and their distribution on a regional scale (100−1000 km).

Experimental Section

The methods used for sample collection, preparation, and fractionation, and those used to measure and calculate the mutagenicity have been described elsewhere (*16, 17*); therefore, summaries are provided here.

Sample Preparation. Annual composite $PM_{2.5}$ samples from three of the five sampling sites (Figure 1) were subjected to detailed chemical characterization. Two sites were in MA: Kenmore Square (KS) in downtown Boston, the largest coastal urban center in New England; and Quabbin State Park (QB), a regional background site in central MA. The third site was in downtown Rochester (RO), an inland, urban center in northern NY. The three sites were located at approximately the same latitude (42° 16′ to 43° 7′), and the distance between the westernmost (RO) and easternmost (KS) sites was ∼640 km; QB was ∼130 km west of KS. During periods of westerly winds, these sites form a west-to-east, downwind transect. Sampling was performed at the three sites simultaneously for 24 h every sixth day for the entire calendar year of 1995. Particles were collected on quartz-fiber filters using a high-volume (∼300 L/min) dichotomous virtual impactor with a size cutoff at ∼3 μm (*18, 19*). Organic carbon (OC) was measured on each filter by a thermal evolution and combustion method (*20*). Results are presented per mass of OC measured on a filter prior to extraction (equivalent organic carbon, or EOC) and per m^3 of air.

The annual composite samples were created by combining 1/24 of each filter collected at a site. The filters were extracted in the dark for 24 h in 100 mL of HPLC-grade dichloromethane (CH_2Cl_2) using a Soxhlet extractor; an additional 24 h of extraction yielded negligible amounts of target compounds. Extracts were concentrated under a gentle stream of N_2, but not to dryness, and then filtered through a 0.2-μm filter. The extracts were fractionated by normal-phase HPLC, using a Beckman Gold HPLC system with an Econosil 10-μm preparatory cyanopropyl (CN) column (250 mm length × 10 mm i.d.) operating at 4 mL/min. CN material has been shown to preserve the mutagenicity of complex mixtures during fractionation (*21*). High-purity hexane, 2-isopropyl alcohol (Omnisolve HPLC grade), and CH_2Cl_2 (Baker ultra-resi) were used as mobile phases, beginning with 95:5 hexane/CH_2Cl_2 for 20 min, ramping to 100% CH_2Cl_2 over 10 min and holding for 10 min, and then ramping to 50:50 CH_2Cl_2/isopropyl alcohol over 10 min and holding for 20 min. Four fractions were created (the cutoff times are shown in parentheses): nonpolar 1 (NP1; 350 s), nonpolar 2 (NP2; 600 s), semipolar (SP; 2650 s), polar (P; 3800 s). Examples of the compounds recovered in each fraction are given elsewhere (*16*). Recovery of PAH using this fractionation method is reported to be 77−110% (*15*). The fractions were then concentrated again under a gentle stream of N_2 prior to GC−MS analysis.

Human Cell Mutation Assay. Mutagenicity was measured using the h1A1v2 human B-lymphoblastoid cell line, a metabolically competent cell line that constitutively expresses CYP1A1 at levels ∼50-fold higher than the basal level and ∼3-fold higher than the fully induced level of the parent cell line (*22*). Whole and fractionated extracts were exchanged into DMSO and tested at 3−5 concentrations in triplicate 12-mL cultures along with two replicates of the positive controls (1.0 μg/mL benzo[*a*]pyrene) and four replicates of the negative controls (0.3 vol % DMSO). The maximum amount of DMSO added to cell cultures during treatment was 0.3 vol %. Exponentially growing cells (1.8×10^6 cells/mL) were incubated for 72 h in the presence of the sample material. Treatment was terminated by centrifuging the cells and resuspending them in fresh medium. One day later, the cultures were counted and diluted up to 2.0×10^5 cells/mL. The cultures were grown for an additional 2 days without dilution to allow for the phenotypic expression of mutations, after which the cultures were plated in 96-well microtiter plates in the presence (mutagenicity) and absence (colony forming efficiency) of the selective agent trifluorothymidine (2 μg/mL). Twenty thousand cells were added per well to the mutagenicity plates ($n = 3$); two cells were added per well to the colony forming efficiency plates ($n = 2$). The plates were incubated for 13 days and scored for the presence of a colony in each well. This assay protocol allows measurement of point mutations (i.e., base-pair additions, deletions, transitions, and transversions) and other events (e.g., nonlethal recombination or chromosomal loss) leading to the loss of heterozygocity at the *thymidine* kinase gene locus. Additional details of the assay are described by Busby et al. (*23, 24*).

The methods used to calculate colony forming efficiency and mutant fraction have been described by Furth et al. (*25*). Relative survival was determined as the ratio of the growth of cells in the treated cultures to the growth of cells in the control cultures from the beginning of treatment until the time of plating. Samples were considered mutagenic if (1) the concentration−response relationship was positive, and (2) the mean mutant fraction (MMF) at one or more concentrations exceeded both the 95% upper confidence limit (UCL) of the concurrent negative controls (calculated using Dunnett's *t*-test) and the 99% UCL of the historical negative controls (38.2×10^{-6}, calculated using a Gamma distribution) (*26*). The mean mutant fraction (±SD) for the negative controls for $n = 600$ independent experiments was $21.4 \times 10^{-6} \pm 7.07 \times 10^{-6}$.

Mutagenicity results are presented in two ways, both of which are based on the induced mutant fraction or IMF (i.e., mutagenicity induced in excess of that produced by the concurrent negative controls, as described elsewhere (*16, 17, 27*)). The first, mutagenic potency, is defined as IMF × 10^6/mg EOC (or IMF × $10^6/\mu$g for neat standards). This measure is normalized to EOC; therefore, the mutagenicity of the particles can be evaluated independently of the effects of atmospheric dilution. Mutagenic potency is equal to the slope of the initial linear portion of the dose-IMF curve, determined based on the fit of a least-squares linear regression line forced through the origin. The second, mutagen density, defined as IMF × $10^6/m^3$ of air, was obtained by multiplying mutagenic potency data by ambient OC concentrations.

The mutagenicity of individual compounds to h1A1v2 cells was calculated using data published elsewhere (*27, 28*). Mutagenicity of individual compounds, expressed as IMF × $10^6/\mu$g, was determined from the slope of a linear regression fit to all replicate measurements. For several compounds the responses at high doses reached a plateau, and so only the initial slope of the dose−response plot was used. This is consistent with the methodology used to determine the mutagenic potency of the whole and fractionated extracts of the $PM_{2.5}$ samples (*16, 17*). The mutagenicity attributable to each mutagen (expressed as a fraction of the total mutagenicity) was calculated by multiplying the mutagenic potency of each known mutagen by its concentration in the sample, then dividing by the mutagenic potency of the unfractionated extracts.

Chemical Analysis. Extracts of the three annual composite samples and their four fractions were analyzed in triplicate on a Hewlett-Packard 6890/5973 gas chromatograph−mass spectrometer (GC−MS) system. The GC was equipped with a Hewlett-Packard HP-5MS capillary column (5% cross-linked phenyl methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness). The MS was operated in the electron impact mode at an energy of 70 eV, a quadrupole temperature of 150 °C, a source temperature of 250 °C, and a transfer line temperature of 280 °C. Sample extracts were analyzed by injecting 1−2 μL into a split/splitless injector port operating at 280 °C in splitless mode, with a continuous flow of helium at 1 mL/min. The oven program was as follows: hold at 50 °C for 1.5 min following injection, ramp at 6 °C min^{-1} to 310 °C, hold at 310 °C for 10 min. Compounds were identified and quantified in scan mode, monitoring for ions with a mass/charge ratio (m/z) between 50 and 350. Retention indices were calculated using 200 as the index for naphthalene, 300 for phenanthrene, 400 for chrysene, and 501.32 for benzo[*ghi*]perylene (*29*). Quantitation limits for individual compounds on this instrument were ∼0.04 ng for PAHs and ∼0.1 ng for other PACs.

Quantitation was based on 19 internal standards and response factors for 40 authentic standards determined with respect to the internal standards (Table 1). Just prior to extraction, the filters were spiked with a solution of the internal standards: 15 deuterated PAHs (Canadian National Research Council (*30*)), 3 deuterated nitro-PAHs (Cambridge Isotopes Laboratory; Andover, MA), and 4-fluoro-1-naphthoic acid (Aldrich Chemical Co.; St. Louis, MO). The internal standards were added in proportion to the organic carbon (EOC) content of each sample, which varied among the three sites (see amounts in ng/mg EOC in Table 1). Site-specific limits of detection were ∼0.1−0.4 ng/mg EOC for PAHs, ∼0.4−1.1 ng/mg EOC for oxy-PAH, and ∼1−2 ng/mg EOC for nitro-PAH; the limit of detection varied among sites due to different amounts of EOC in each sample. The compounds used as internal standards were chosen to encompass a wide range of GC retention times, and so that each HPLC fraction would contain at least one internal standard. Response factors for compounds other than the 40 authentic standards were assigned based on response factors of chemically similar standards (e.g., benzo[*e*]pyrene and perylene were assigned the response factor for benzo[*a*]pyrene). Identification of target compounds was confirmed using both retention indices and mass spectra from authentic standards, or from the literature when authentic standards were not available. Methyl- and dimethyl-phenanthrenes were identified as reported by Benner et al. (*31, 32*); methylfluoranthene and methylpyrene isomers were identified as reported by Hilpert (*33*). Benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene coeluted on the HP-5MS column, as did 2- and 3-nitrofluoranthene; thus, these compounds were reported as the sum of the coeluting isomers (Table 2). Particular care was given to properly identifying PAHs with molecular weight of 302 amu (6-ring, $C_{24}H_{14}$) because their genotoxicity varies widely, from nonmutagenic to highly mutagenic (*27*). The MW 302 amu PAHs were identified based on the m/z 302 peaks reported for $PM_{2.5}$ samples from KS (*34*). For the purpose of calculating attributed mutagenicity, the coeluting MW 302 amu PAHs were assumed to be distributed according to the concentrations reported for SRM 1649a (*35*).

TABLE 1. Internal and Target Compound Standards[a]

	standard used to determine response factors	ISTD ng/mg EOC
*1	naphthalene	230
*2	acenaphthylene	46
*3	acenaphthene	23
*4	fluorene	46
*5	phenanthrene	182
*6	anthracene	25
*7	fluoranthene	230
*8	pyrene	184
9	8-methylfluoranthene	
10	benzo[*b*]fluorene	
*11	benz[*a*]anthracene	115
*12	chrysene	115
13	5-methylchrysene	
*14	benzo[*b*]fluoranthene	69
15	benzo[*k*]fluoranthene	
*16	benzo[*a*]pyrene	69
17	1-methylbenzo[*a*]pyrene	
*18	dibenz[*ah*]anthracene	23
19	indeno[123-*cd*]pyrene	
*20	benzo[*ghi*]perylene	46
*21	coronene	16
*22	2-nitrofluorene	115
23.	9-nitroanthracene	
24.	1,8-dinitronaphthalene	
25.	vanillin	
26.	1-naphthol	
27.	9-fluorenone	
28.	anthrone	
29.	9,10-anthracenequinone	
30.	1,8-naphthalic anhydride	
31.	3-nitrofluoranthene	
*32	1-nitropyrene	115
33	7H-benz[*de*]anthracene-7-one	
34	benz[*a*]anthracene-7,12-quinone	
35	7-nitrobenz[*a*]anthracene	
*36	6-nitrochrysene	115
37	1,8-dinitropyrene	
38	6-nitrobenzo[*a*]pyrene	
39	4-fluoro-1-naphthoic acid (ISTD)	115
40	2-naphthoic acid	

[a] Deuterated forms of the compounds marked with an asterisk (*) and the 4-fluoro-1-naphthoic acid were added to the filters before extraction in the amounts listed (ng/mg EOC) to serve as internal standards. Response factors were developed for each of the 40 listed compounds with respect to its deuterated form or the deuterated internal standard listed above it.

TABLE 2. Concentrations of Organic Compounds Measured in Annual Composites of $PM_{2.5}$ from Rochester (RO), Quabbin Summit (QB), and Kenmore Square (KS)

mut.[a]	compound name[b]	formula (MW)	RO (stdv)	QB (stdv)	KS (stdv)	RO (stdv)	QB (stdv)	KS (stdv)	ID[d]	ret. index
			ng/mg EOC [c]			pg m^{-3} [c]				
	Nonpolar Fraction 1 (NP1)									
+	acenaphthylene	$C_{12}H_8$ (152)	11 (1)	5 (1)	6.3 (0.5)	38 (3)	14 (3)	36 (3)	a	248.47
	acenaphthene	$C_{12}H_{10}$ (154)	8 (2)	7.7 (0.1)	7.0 (0.9)	27 (7)	21 (0.3)	40 (5)	a	254.07
	fluorene	$C_{13}H_{10}$ (166)	5.0 (0.3)	3.3 (0.5)	6.5 (0.9)	17 (1)	9 (1)	37 (5)	a	270.32
	dibenzothiophene	$C_{12}H_8S$ (184)	11 (1)	5.1 (0.7)	14 (0.5)	36 (3)	14 (2)	81 (3)	b	296.06
-	phenanthrene	$C_{14}H_{10}$ (178)	47 (1)	19 (1.4)	50 (1)	160 (3)	52 (4)	280 (6)	a	300.00
	anthracene	$C_{14}H_{10}$ (178)	15 (0.4)	5.4 (0.3)	11 (0.2)	49 (1)	15 (0.8)	64 (1)	a	301.68
	3-methylphenanthrene	$C_{15}H_{12}$ (192)	6.7 (0.2)	2.8 (0.1)	28 (0.4)	23 (0.7)	8 (0.3)	160 (2)	B	318.93
	2-methylphenanthrene	$C_{15}H_{12}$ (192)	11 (0.3)	4.5 (0.1)	40 (1)	37 (1)	12 (0.3)	230 (6)	B	319.85
	9-, & 4-methylphenanthrene	$C_{15}H_{12}$ (192)	6 (1.3)	2.7 (0.2)	19 (1)	20 (4)	7.3 (0.5)	110 (6)	B	322.92
+	1-methylphenanthrene	$C_{15}H_{12}$ (192)	6.3 (0.3)	3.4 (0.5)	19 (4)	21 (1)	9 (2)	110 (23)	B	323.79
	total C1-PAH178	$C_{15}H_{12}$ (192)	30 (1.4)	13 (0.6)	110 (4)	100 (5)	36 (2)	600 (23)		
	cyclopenta[*def*]phenanthrene	$C_{15}H_{10}$ (190)	6 (1.2)	10 (13)	10 (3)	20 (4)	27 (36)	57 (17)	b	322.64
	3-ethylphenanthrene	$C_{16}H_{14}$ (206)	1.6 (0.2)	I. D.	7 (0.4)	5.5 (0.7)	I. D.	40 (2)	B	333.70
	2 & 9-ethylphenanthrene + 3,6-dimethylphenanthrene	$C_{16}H_{14}$ (206)	4.6 (0.3)	2.2 (0.8)	32 (2)	16 (1)	6 (2)	180 (11)	B	336.46
	2,6-dimethylphenanthrene	$C_{16}H_{14}$ (206)	5.9 (0.6)	2.4	46 (3)	20 (2)	6.5	260 (17)	B	337.96
	2,7-dimethylphenanthrene	$C_{16}H_{14}$ (206)	3.5 (0.3)		34 (0.7)	12 (1)		190 (4)	B	338.49
	1,3-, 2,10-, 3,9-, & 3,10-dimethylphenanthrenes	$C_{16}H_{14}$ (206)	11 (0.7)	2.6 (0.9)	95 (8)	37 (2)	7 (2)	540 (45)	B	340.79
	1,6- & 2,9-dimethylphenanthrene	$C_{16}H_{14}$ (206)	5.1 (0.6)	2.0 (0.2)	55 (2)	17 (2)	5.4 (0.5)	310 (11)	B	341.75
	1,7-dimethylphenanthrene	$C_{16}H_{14}$ (206)	4.2 (0.6)	2.1 (0.2)	35 (2)	14 (2)	5.7 (0.5)	200 (9)	B	342.60
	2,3-dimethylphenanthrene	$C_{16}H_{14}$ (206)	3 (5)	2.2	22 (1)	10 (17)	6	120 (6)	B	343.92
	1,9- & 4,9-dimethylphenanthrene	$C_{16}H_{14}$ (206)	2.1 (1.4)		15 (0.1)	7 (5)		84 (0.6)	B	344.26
	1,8-dimethylphenanthrene	$C_{16}H_{14}$ (206)	N. D.	N. D.	8 (2)	N. D.	N. D.	45 (11)	B	
	total C2-PAH178	$C_{16}H_{14}$ (206)	46 (4)	12 (0.2)	340 (52)	160 (14)	32 (0.5)	1,900 (300)		
	C3-PAH178a	$C_{17}H_{16}$ (220)	6.5 (0.5)	I. D.	43 (4)	22 (2)	I. D.	250 (23)	c	357.31
	C3-PAH178b	$C_{17}H_{16}$ (220)	8 (1)	3.7 (0.7)	50 (3)	27 (3)	10 (2)	280 (17)	c	358.43
	C3-PAH178c	$C_{17}H_{16}$ (220)	3.6 (0.5)	I. D.	17 (0.9)	12 (2)	I. D.	98 (5)	c	360.04
	C3-PAH178d	$C_{17}H_{16}$ (220)	4.8 (0.3)	I. D.	28 (3)	16 (1)	I. D.	160 (17)	c	361.09
	C3-PAH178e	$C_{17}H_{16}$ (220)	2.8 (0.1)	N. D.	10 (6)	9.5 (0.3)	N. D.	57 (34)	c	362.75
	C3-PAH178f	$C_{17}H_{16}$ (220)	1.6 (0.9)	N. D.	6.9 (0.6)	5 (3)	N. D.	39 (3)	c	364.22
	C3-PAH178g	$C_{17}H_{16}$ (220)	2.1 (0.2)	N. D.	10 (0.6)	7.2 (0.7)	N. D.	60 (3)	c	365.05
	C3-PAH178h	$C_{17}H_{16}$ (220)	1.4 (0.3)	N. D.	5.1 (0.4)	5 (1)	N. D.	29 (2)	c	366.17
	C3-PAH178i	$C_{17}H_{16}$ (220)	1.9 (1.1)	N. D.	7.6 (4)	6 (4)	N. D.	45 (23)	c	369.90
	total C3-PAH178	$C_{17}H_{16}$ (220)	36 (6)	7.0 (0.2)	270 (40)	120 (20)	19 (0.5)	1,500 (230)		
	total C4-PAH178	$C_{18}H_{18}$ (234)	26 (4.5)	6.0 (0.5)	120 (9)	90 (15)	16 (1)	700 (50)	c	369.20
	retene (7-isopropyl-1-methylphenanthrene)	$C_{18}H_{18}$ (234)	4.0 (0.5)	5.2 (0.4)	7.3 (1.1)	14 (2)	14 (1)	41 (6)	c	367.31
	Nonpolar Fraction 2 (NP2)									
-	fluoranthene	$C_{16}H_{10}$ (202)	110 (1.5)	45 (0.1)	100 (0.6)	360 (5)	120 (0.3)	590 (3)	a	344.85
-	pyrene	$C_{16}H_{10}$ (202)	85 (1.3)	37 (1)	130 (1)	290 (4)	100 (3)	740 (6)	a	352.67
+	8-methylfluoranthene	$C_{17}H_{12}$ (216)	18 (0.6)	6.0 (0.5)	34 (0.5)	63 (2)	16 (1)	190 (3)	a	362.76
	1+3+7-methylfluoranthene	$C_{17}H_{12}$ (216)	43 (0.5)	22 (0.8)	86 (3)	150 (2)	58 (2)	490 (17)	H	366.64
-	benzo[*b*]fluorene	$C_{17}H_{12}$ (216)	16 (0.5)	1.0 (0.6)	25 (1)	53 (2)	3 (2)	140 (6)	a	369.16
	2-methylpyrene	$C_{17}H_{12}$ (216)	12 (1)	5.5 (0.1)	38 (2)	41 (3)	15 (0.3)	220 (11)	H	370.10
	4-methylpyrene	$C_{17}H_{12}$ (216)	12 (0.9)	6.2 (0.4)	40 (1)	41 (3)	17 (1)	230 (6)	H	373.41
	1-methylpyrene	$C_{17}H_{12}$ (216)	9.2 (0.5)	5.6 (0.3)	32 (0.9)	31 (2)	15 (0.8)	180 (5)	H	374.43
	total C1-PAH202	$C_{17}H_{12}$ (216)	95 (2)	45 (1)	230 (4)	320 (7)	120 (3)	1,300 (23)		
	total C2-PAH202	$C_{18}H_{14}$ (230)	10 (1.4)	2.5(0.01)	38 (1.2)	34 (5)	6.8 (0.1)	220 (7)	c	
-	benz[*ghi*]fluoranthene	$C_{18}H_{10}$ (226)	45 (0.4)	18 (0.4)	74 (2)	150 (1)	48 (1)	420 (11)	b	391.02
+	benzo[*c*]phenanthrene	$C_{18}H_{12}$ (228)	9.0 (0.9)	3.8 (0.2)	13 (0.7)	31 (3)	10 (0.5)	75 (4)	b	391.11
	benzonaphthothiophene a	$C_{16}H_{10}S$ (234)	21 (1)	7.9 (0.3)	29 (1)	72 (3)	22 (0.8)	170 (6)	b	389.50
	benzonaphthothiophene b	$C_{16}H_{10}S$ (234)	5.2 (0.2)	3.0 (0.4)	5.9 (0.3)	18 (0.7)	8.2 (1)	34 (2)	b	392.81
	benzonaphthothiophene c	$C_{16}H_{10}S$ (234)	7.1 (0.6)	4.0 (0.3)	8.4 (0.7)	24 (2)	11 (0.8)	48 (4)	b	395.69
+++	cyclopenta[*cd*]pyrene	$C_{18}H_{10}$ (226)	2.2 (0.4)	0.7 (0.4)	3.5 (0.2)	8 (1)	2 (1)	20 (1)	b	397.93
++	benz[*a*]anthracene	$C_{18}H_{12}$ (228)	32 (1)	15 (0.1)	49 (0.5)	110 (3)	41 (0.3)	280 (3)	a	398.60
++	chrysene & triphenylene	$C_{18}H_{12}$ (228)	98 (1)	48 (0.3)	110 (1)	330 (3)	130 (1)	620 (5)	a	400.00
++	naphthacene	$C_{18}H_{12}$ (228)	3.1 (0.2)	0.9 (0.4)	2.7 (0.3)	11 (0.7)	2.5 (1)	15 (2)	b	403.15
	methyl-PAH226a	$C_{19}H_{12}$ (240)	7.0 (0.3)	4.7 (0.4)	8.9 (0.2)	24 (1)	13 (1)	51 (1)	c	420.43
	methyl-PAH226b	$C_{19}H_{12}$ (240)	9.5 (0.5)	5.8 (0.2)	11 (0.6)	32 (2)	16 (0.5)	61 (3)	c	421.80
	methyl-PAH226c	$C_{19}H_{12}$ (240)	9.5 (0.3)	5.9 (0.2)	13 (0.6)	32 (1)	16 (0.5)	74 (3)	c	422.66
	methyl-PAH228a	$C_{19}H_{14}$ (242)	21 (1.0)	11 (1)	46 (0.6)	72 (3)	30 (3)	260 (3)	c	417.21
	methyl-PAH228b	$C_{19}H_{14}$ (242)	12 (0.3)	7.2 (0.6)	24 (0.3)	41 (1)	20 (2)	140 (2)	c	418.67
+++	5-methylchrysene	$C_{19}H_{14}$ (242)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	419.88
	methyl-PAH228c	$C_{19}H_{14}$ (242)	6 (0.6)	4.8 (0.3)	12 (0.5)	20 (2)	13 (0.8)	68 (3)	c	420.54
	methyl-PAH228d	$C_{19}H_{14}$ (242)	8 (0.3)	6 (0.5)	13 (0.6)	27 (1)	16 (1)	74 (3)	c	422.66
	total C1-PAH228	$C_{19}H_{14}$ (242)	47 (2)	29 (2)	96 (3)	160 (7)	79 (5)	540 (17)		
	total C2-PAH228	$C_{20}H_{16}$ (256)	30 (2)	17 (1)	71 (5)	100 (7)	46 (3)	400 (28)	c	

TABLE 2. Continued

mut.[a]	compound name[b]	formula (MW)	RO (stdv)	QB (stdv)	KS (stdv)	RO (stdv)	QB (stdv)	KS (stdv)	ID[d]	ret. index
			ng/mg EOC [c]			pg m^{-3} [c]				
	Nonpolar Fraction 2 (NP2), Continued									
++	benzo[*b*+*j*+*k*]fluoranthene	$C_{20}H_{12}$ (252)	140 (1)	82 (0.7)	130 (2)	470 (3)	220 (2)	740 (11)	a	443.19
	PAH 252	$C_{20}H_{12}$ (252)	8.3 (0.2)	6.0 (0.4)	10 (0.6)	28 (0.7)	16 (1)	59 (3)	c	447.14
+	benzo[*e*]pyrene	$C_{20}H_{12}$ (252)	69 (1)	38 (1)	70 (0.8)	240 (3)	100 (3)	400 (5)	b	453.12
+++	benzo[*a*]pyrene	$C_{20}H_{12}$ (252)	34 (0.4)	20 (0.6)	37 (1)	120 (1)	55 (2)	210 (6)	a	454.87
-	perylene	$C_{20}H_{12}$ (252)	14 (2)	9 (2)	12 (2)	48 (7)	24 (5)	68 (11)	b	457.93
+++	1-methylbenzo[*a*]pyrene	$C_{21}H_{14}$ (266)	10 (0.9)	6 (2)	15 (1)	35 (3)	16 (5)	85 (6)	a	473.70
	total C1-PAH252	$C_{21}H_{14}$ (266)	61 (10)	38 (1)	86 (4)	210 (34)	100 (3)	490 (23)	b	
	Semipolar Fraction (SP)									
	PAH 276a	$C_{22}H_{12}$ (276)	4.2 (0.8)	3.0 (0.6)	5.2 (0.2)	14 (3)	8 (2)	30 (1)	c	485.15
	indeno[123-*cd*]fluoranthene	$C_{22}H_{12}$ (276)	30 (3)	15 (2)	33 (3)	100 (10)	41 (5)	190 (17)	b	490.85
++	dibenz[*aj*]anthracene	$C_{22}H_{14}$ (278)	17 (0.9)	12 (0.5)	18 (0.2)	57 (3)	32 (1)	100 (1)	c	490.89
++	indeno[123-*cd*]pyrene	$C_{22}H_{12}$ (276)	81 (3)	40 (1)	86 (6)	280 (10)	110 (3)	490 (34)	a	493.65
++	dibenz[*ah*]anthracene	$C_{22}H_{14}$ (278)	14 (0.3)	11 (0.3)	16 (2)	46 (1)	31 (0.8)	91 (11)	a	494.91
	PAH 278a	$C_{22}H_{14}$ (278)	9.5 (0.2)	8.0 (0.3)	10 (0.6)	32 (0.7)	22 (0.8)	57 (3)	c	497.96
	PAH 278b	$C_{22}H_{14}$ (278)	13 (0.4)	10 (0.4)	12 (1)	43 (1)	28 (1)	68 (6)	c	499.05
++	benzo[*ghi*]perylene	$C_{22}H_{12}$ (276)	87 (1)	28 (1)	86 (4)	300 (3)	76 (3)	490 (23)	a	501.32
	PAH 276b	$C_{22}H_{12}$ (276)	5 (1)	0.4 (0.1)	3.8 (0.4)	17 (3)	1.1 (0.3)	22 (2)	c	505.28
	PAH 302a	$C_{24}H_{14}$ (302)	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	J	543.06
++,+	N12kF, N12bF	$C_{24}H_{14}$ (302)	16 (3)	11 (2)	13 (4)	55 (10)	30 (5)	75 (23)	J	538.70
++,++	N23bF, DBbkF	$C_{24}H_{14}$ (302)	11 (2)	6.8 (0.6)	8 (2)	38 (7)	19 (2)	45 (11)	J	541.53
	PAH-302d	$C_{24}H_{14}$ (302)	8.7 (0.3)	5.2 (0.7)	7 (1)	30 (1)	14 (2)	40 (6)	J	543.06
+++	naphtho[2,3-*e*]pyrene	$C_{24}H_{14}$ (302)	2.8	N. D.	4.5	10	N. D.	26	J	550.81
-	coronene	$C_{24}H_{12}$ (300)	42 (5)	17 (4)	42 (13)	140 (17)	46 (11)	240 (75)	a	554.42
+++	dibenzo[*ae*]pyrene	$C_{24}H_{14}$ (302)	5.2 (0.3)	4 (2)	6 (2)	18 (1)	11 (5)	34 (11)	J	555.26
++,++	N21aP, DBeIP	$C_{24}H_{14}$ (302)	6 (1)	3.6 (0.2)	7 (2)	20 (3)	9.8 (0.5)	40 (11)	J	556.38
+++	naphtho[2,3-*a*]pyrene	$C_{24}H_{14}$ (302)	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	J	
+++	dibenzo[*ai*]pyrene	$C_{24}H_{14}$ (302)	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	J	
	Nitro-PAH									
-	2-nitrofluorene	$C_{13}H_9NO_2$ (211)	47 (4)	12 (0.6)	55 (6)	160 (14)	34 (1)	310 (34)	a	351.77
+	9-nitroanthracene	$C_{14}H_9NO_2$ (223)	12 (5)	N. D.	13	41 (17)	N. D.	74	a	356.50
	1,8-dinitronaphthalene	$C_{10}H_6N_2O_4$ (218)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	360.85
+	2+3-nitrofluoranthene	$C_{16}H_9NO_2$ (247)	26 (10)	14 (3)	N. D.	90 (34)	38 (8)	N. D.	a	412.45
+	1-nitropyrene	$C_{16}H_9NO_2$ (247)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	421.53
	7-nitrobenz[*a*]anthracene	$C_{18}H_{11}NO_2$ (273)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	448.88
	6-nitrochrysene	$C_{18}H_{11}NO_2$ (273)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	460.49
++	1,8-dinitropyrene	$C_{16}H_8N_2O_4$ (292)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	484.04
	6-nitrobenzo[*a*]pyrene	$C_{16}H_9NO_2$ (297)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	501.46
	PAH Ketones and Quinones									
	iso-indole dione	$C_8H_5NO_2$ (147)	49 (17)	N. D.	28 (8)	170 (58)	N. D.	160 (45)	d	251.70
	methylnaphthalene dione isomer	$C_{11}H_8O_2$ (172)	17 (1)	5 (2)	4 (3)	58 (3)	14 (5)	23 (17)	d	259.79
	9-fluorenone	$C_{13}H_8O$ (180)	44 (4)	18 (3)	44 (5)	150 (14)	49 (8)	250 (28)	a	294.03
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	d	307.38
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	9 (2)	I. D.	25 (5)	31 (7)	I. D.	140 (30)	d	313.20
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	16 (0.4)	I. D.	44 (9)	53 (1)	I. D.	250 (51)	d	315.61
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	I. D.	I. D.	4 (5)	I. D.	I. D.	23 (28)	d	318.15
+	phenalenone	$C_{13}H_8O$ (180)	100 (9)	32 (6)	230 (9)	360 (31)	87 (16)	1,300 (50)	c	320.74
	anthrone	$C_{14}H_{10}O$ (194)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	328.09
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	14 (19)	I. D.	21 (12)	48 (65)	I. D.	120 (70)	d	330.44
	9,10-anthracenequinone	$C_{14}H_8O_2$ (208)	270 (20)	70 (2)	220 (4)	920 (70)	190 (5)	1,200 (23)	a	331.22
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	I. D.	I. D.	16 (1)	I. D.	I. D.	91 (6)	d	338.81
	phenanthrenone/phenanthrenol isomer	$C_{14}H_{10}O$ (194)	I. D.	I. D.	28 (15)	I. D.	I. D.	160 (85)	d	339.91
-	4*H*-cyclopenta[*def*]phenanthrenone	$C_{15}H_8O$ (204)	9.0 (0.6)	N. D.	27 (5)	31 (2)	N. D.	150 (28)	e	342.64
	methylanthracenequinone isomer	$C_{15}H_{10}O_2$ (222)	38 (12)	N. D.	68 (8)	130 (41)	N. D.	390 (45)	c	347.26
	methylanthracenequinone isomer	$C_{15}H_{10}O_2$ (222)	150 (4)	37 (36)	150 (11)	500 (14)	100 (98)	850 (62)	c	351.75
	11*H*-benzo[*a*]fluoren-11-one	$C_{17}H_{10}O$ (230)	180 (28)	50 (5)	260 (27)	610 (95)	140 (14)	1,500 (150)	e	386.04
	7*H*-benzo[*c*]fluoren-7-one	$C_{17}H_{10}O$ (230)	32 (8)	6 (8)	81 (9)	110 (27)	16 (22)	460 (51)	e	390.28
	11*H*-benzo[*b*]fluoren-11-one	$C_{17}H_{10}O$ (230)	160 (25)	53 (8)	240 (29)	560 (85)	140 (22)	1,300 (160)	e	393.84
+	7*H*-benz[*de*]anthracen-7-one	$C_{17}H_{10}O$ (230)	120 (6)	90 (11)	230 (14)	420 (20)	250 (30)	1,300 (80)	a	405.44
-	7,12-benz[*a*]anthracenequinone	$C_{18}H_{10}O_2$ (258)	86 (4)	32 (2)	82 (6)	290 (14)	87 (5)	460 (35)	a	419.09
-	5,12-naphthacenequinone	$C_{18}H_{10}O_2$ (258)	14 (6)	4 (2)	11 (4)	48 (20)	11 (5)	62 (23)	e	430.57
++	6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$ (254)	180 (20)	92 (6)	220 (25)	620 (70)	250 (16)	1,300 (140)	e	454.70

TABLE 2. Continued

mut.[a]	compound name[b]	formula (MW)	RO (stdv)	QB (stdv)	KS (stdv)	RO (stdv)	QB (stdv)	KS (stdv)	ID[d]	ret. index
			ng/mg EOC [c]			pg m^{-3} [c]				
	Other Oxy-PAC									
++	anthanthrenequinone	$C_{22}H_{10}O_2$ (306)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	c	550.62
	vanillin	$C_8H_8O_3$ (152)	230 (1)	190 (83)	46 (13)	790 (3)	520 (220)	260 (75)	a	240.45
	1-naphthol	$C_{10}H_8O$ (144)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	a	259.66
	phthalic anhydride (isobenzofurandione)	$C_8H_4O_3$ (148)	27 (2)	48 (2)	41 (15)	92 (7)	130 (5)	230 (85)	c	225.33
	phthalide (1(3H)-isobenzofuranone)	$C_8H_6O_2$ (134)	220 (56)	270 (66)	110 (37)	740 (190)	730 (180)	630 (210)	d	231.37
	methylphthalide isomer	$C_9H_8O_2$ (148)	57 (8)	43 (25)	25 (5)	200 (27)	120 (70)	140 (28)	d	251.10
	methylphthalide isomer	$C_9H_8O_2$ (148)	110 (17)	77 (37)	69 (11)	360 (60)	210 (100)	390 (62)	d	253.46
	1,3- or 2,4-benzene-dicarboxylic acid, dimethyl ester	$C_{10}H_{10}O_4$ (194)	1,100 (117)	N. D.	N. D.	3,900 (400)	N. D.	N. D.	c	256.92
-	1,2-naphthalic anhydride	$C_{12}H_6O_3$ (198)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	e	310.04
	hydroxyfluorenone/ xanthone isomer	$C_{13}H_8O_2$ (196)	83 (7)	49 (5)	93 (2)	280 (24)	130 (14)	530 (11)	c	313.59
	xanthone	$C_{13}H_8O_2$ (196)	380 (27)	180 (150)	380 (24)	1,300 (90)	480 (410)	2,200 (140)	b	328.31
-	1,8-naphthalic anhydride	$C_{12}H_6O_3$ (198)	120 (22)	80 (0.5)	210 (45)	400 (75)	220 (1)	1,200 (260)	a	342.17
	3-nitrobenzanthrone	$C_{17}H_9O_3N$ (230)	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	d	~490
			Polar Fraction (P)							
	benzoic acid	$C_7H_6O_2$ (122)	370 (96)	200 (30)	160 (17)	1,300 (330)	560 (82)	930 (95)	c	199.20
	benzene dicarboxylic acid	$C_8H_6O_4$ (166)	1,800 (190)	2,600 (570)	1,800 (790)	6,100 (650)	7100 (1600)	10000 (4500)	c	225.33
	hydroxy-methyl-benzoic acid	$C_8H_8O_3$ (152)	430 (80)	290 (86)	220 (140)	1,500 (275)	780 (230)	1,300 (795)	d	231.37
	vanillin	$C_8H_8O_3$ (152)	32 (7)	40 (30)	52 (38)	110 (24)	110 (82)	300(220)	a	240.45
	methyl benzene dicarboxylic acid isomer (detected as 4-methyl isobenzofurandione)	$C_9H_8O_4$ (180)	130 (8)	170 (57)	95 (4)	440 (30)	460 (160)	540 25)	c	241.23
	4-methyl, 1,2-benzene dicarboxylic acid isomer	$C_9H_8O_4$ (180)	270 (11)	350 (100)	290 (19)	910 (38)	940 (270)	1,700 (110)	c	247.15
	2-naphthoic acid	$C_{11}H_8O_2$ (172)	94 (18)	26 (22)	210 (22)	320 (60)	71 (60)	1,200 (120)	a	289.55
	1,2-naphthalene dicarboxylic acid (detected as 1,2-naphthalic anhydride)	$C_{12}H_8O_4$ (216)	54 (11)	28 (16)	110 (20)	180 (38)	76 (44)	640 (110)	e	310.04
	1,8-naphthalene dicarboxylic acid (detected as 1,8-naphthalic anhydride)	$C_{12}H_8O_4$ (216)	110 (6)	65 (20)	150 (0.1)	390 (20)	180 (54)	840 (1)	a	342.17
	phenanthrene dicarboxylic acid isomer (detected as the anhydride)	$C_{16}H_8O_3$ (248)	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	d	395.81
	phenanthrene dicarboxylic acid isomer (detected as the anhydride)	$C_{16}H_8O_3$ (248)	8	N. D.	30 (9)	30	N. D.	170 (50)	d	435.80

[a] Compounds marked with a plus (+) are weakly mutagenic, with specific potency up to IMF $\times$ 10^6/μg; those with two plusses (++) are mutagenic, specific potency 1−25 $\times$ IMF $\times$ 10^6/μg; three plusses (+++) are highly mutagenic, specific potency above 25 $\times$ IMF $\sim$ 10^6/μg; and with a minus sign (−) are not mutagenic to h1A1v2 cells (*27, 28*). Compounds that are not marked in column 2 have not been tested. [b] Benzo[*b*+*j*+*k*]fluoranthene are the sum of benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene, which coeluted on the HP-5MS column. 2+3-nitrofluoranthene are the sum of 2-nitrofluoranthene and 3-nitrofluoranthene, which also coeluted; 2-nitrofluoranthene is generally the more abundant isomer in ambient samples. Abbreviations: dibenzo[*bk*]fluoranthene (DBbkF), naphtho[1,2-*k*]fluoranthene (N12kF), naphtho[1,2-*b*]fluoranthene (N12bF), naphtho[2,3-*b*]fluoranthene (N23bF), dibenzo[*el*]pyrene (DBelP), naphtho[2,1-*a*]pyrene (N21aP). [c] Units are ng per mg (ppm) of equivalent organic carbon (EOC), defined as the amount of organic carbon measured on the filters before extraction. pg/m^3 concentrations were obtained by multiplying ng/mg EOC concentrations by the annual average ambient concentrations of OC at each of the sites ($\pm$ standard error of the mean): 3.4 ($\pm$0.04) μg EOC/m^3 at RO, 2.7 ($\pm$0.04) μg EOC/m^3 at QB, and 5.7 ($\pm$0.06) μg EOC/m^3 at KS (*16*). N. D. = not detected. I. D. = identified but not quantified. Site-specific limits of detection were ~0.1−0.4 ng/mg EOC for PAHs, ~0.4−1.1 ng/mg EOC for oxy-PAH, and ~1−2 ng/mg EOC for nitro-PAH. [d] Identification of compounds based on (a) retention index, mass spectrum, and response factor (RF) of authentic standard; (b) retention index and spectrum of authentic standard, and RF for compound of similar MW and structure; (c) relative retention time and spectrum from library using RF for compound of similar MW and structure; (d) relative retention time and spectrum from library using RF for compound with similar structure; (e) retention index and spectrum for authentic standard using same column and a different GC oven program; (B) ID based on Benner et al. (*31*), RF for phenanthrene; (H) ID based on Hilpert (*33*), RF for pyrene; (J) retention index and identification from Allen et al. (*34*), RF of coronene.

TABLE 3. Particulate PAC Concentrations Reported in Other Studies

location	compounds[a]	results	our results
	Urban Sites		
Chicago, IL (*36*)	∑16PAH	14 ng m^{-3}	2.9−5.3 ng m^{-3} (RO−KS)
Munich, Germany (*37*)	∑7PAH [a]	1.2−5.7 ng m^{-3}	1.9−3.4 ng m^{-3} (RO−KS)
	9-fluorenone	220−460 pg m^{-3}	150−250 pg m^{-3} (RO−KS)
	benzofluorenones	0.5−1.5 ng m^{-3}	0.9−1.2 ng m^{-3} (RO−KS)
	1,8-naphthalic anhydride	1.3−2.7 ng m^{-3}	0.4−1.2 ng m^{-3} (RO−KS)
Naples, Italy (*38*)	∑17PAH	8−74 ng m^{-3}	1.1−5.4 ng m^{-3} (RO−KS)
	cyclopenta[*cd*]pyrene	20−4,600 pg m^{-3}	8−20 pg m^{-3} (RO−KS)
Rome, Italy (*39*)	∑7PAH[a]	6.0 ng m^{-3}	0.6−2.2 ng m^{-3} (RO−KS)
Mumbai, India (*40*)	∑7PAH[a]	25−39 ng m^{-3}	0.8−4 ng m^{-3} (RO−KS)
Copenhagen, Denmark (*41*)	∑7PAH[a]	5−39 ng m-3	1.7−2.9 ng m-3 (RO−KS)
Copenhagen, Denmark (*42*)	∑13PAH	44 ng m-3	2.6−4.8 ng m-3 (RO−KS)
New Brunswick, NJ (*43*)	∑5PAH[a]	0.71 ng m-3	1.0−2.0 ng m-3 (RO−KS)
Kenmore Square, Boston (*44,45*)	∑9PAH	34 ng m-3	4.8 ng m^{-3} (KS)
	6H-benzo[cd]pyren-6-one	1.3 ng m-3	1.3 ng m^{-3} (KS)
	7H-benz[de]anthracen-7-one	1.2 ng m-3	1.3 ng m^{-3} (KS)
	1,8-naphthalic anhydride	1.8 ng m-3	1.2 ng m^{-3} (KS)
Los Angeles, CA (*14*)	∑5PAH	4.2 ng m^{-3}	1.2−1.9 ng m^{-3} (RO−KS)
	6*H*-benzo[*cd*]pyren-6-one	1.2 ng m^{-3}	0.6−1.3 ng m^{-3} (RO−KS)
	Cyclopenta[*cd*]pyrene	200 pg m^{-3}	8−20 pg m^{-3} (RO−KS)
	Rural Sites		
Quabbin Summit, MA (*44,45*)	∑9PAH	1.2 ng m^{-3}	1.0 ng m^{-3} (QB)
Lake Superior (*36*)	∑16PAH	160−410 pg m^{-3}	1,100 pg m^{-3} (QB)
Risø, Denmark (*42*)	∑13PAH	6.4 ng m^{-3}	1.0 ng m^{-3} (QB)
Sandy Hook, New Jersey (*43*)	∑5PAH[a]	0.3 ng m^{-3}	0.4 ng m^{-3} (QB)

[a] The summed concentrations of PAHs (∑PAH) reflect those reported in the cited references, and are not necessarily the same for the different studies. For example, ∑7 PAH in the Rome study are not the same 7 PAHs as in the Mumbai study. To facilitate comparison of our results with those in the cited studies, the PACs in the cited studies were used.

Every positively identified peak in each chromatogram was identified and integrated manually using HP ChemStation software. After daily autotuning, GC−MS performance was checked each day by running an extract of airborne particles followed by a solution of the internal and target compound standards. CH_2Cl_2 blanks (2 μL) were run before every sample and standard to eliminate carryover. Concentrations of target compounds in fractionated and unfractionated composites were blank-corrected using identically collected and fractionated field blank samples from each site.

Results and Discussion

Quantification of PACs and Other Organic Compounds. Over 150 compounds were measured in the annual composite samples and each of their fractions (Table 2). Concentrations of the target compounds in the fractions confirmed those measured in the unfractionated extracts. The first nonpolar fraction (NP1) contained many aliphatic organic compounds, unsubstituted PAHs up to MW 178 amu, and alkylated PAHs up to C_4-anthracenes and C_4-phenanthrenes, as well as other relatively low molecular weight PACs such as dibenzothiophene. The second nonpolar fraction (NP2) contained unsubstituted PAHs of MW 178−278 amu and their alkylated derivatives, and other PACs such as benzonaphthothiophenes. The semipolar fraction (SP) contained nonpolar PAHs of MW 278−302 amu including indeno[1,2,3-*cd*]pyrene, indeno-[1,2,3-*cd*]fluoranthene, benzo[*ghi*]perylene, coronene, dibenzopyrenes, naphthopyrenes, and naphthofluoranthenes. Some nitro-PAHs were present in this fraction, as well as many oxygenated-PACs including PAH ketones, quinones, and dicarboxylic acid anhydrides. The polar fraction (P) contained hydroxy-PAHs and PAH carboxylic and dicarboxylic acids. Some nonaromatic compounds such as C_8−C_{19} alkanoic acids were also detected in this fraction, but are not listed in Table 2.

In general, concentrations of PAH and oxy-PAH measured at KS, QB, and RO were within the range of measurements reported for other rural and urban sites (Table 3) (*14, 36*−*45*). Allen et al. (*44, 45*) quantified PAHs in size-segregated aerosols collected at both KS and QB in the summer of 1994. We found that the sum of the concentrations of nine PAHs was nearly 10-fold lower in KS than that reported by Allen et al. This discrepancy may be attributable to differences in the location of sampling equipment: our equipment was located on top of a building ∼9 m above street level, while Allen et al. located their equipment in the median of a heavily trafficked avenue, about 300 m to the east of our site. In contrast, at QB the PAH levels we measured were similar to those reported by Allen et al., who collected their samples at the same location as we did. PAH concentrations at QB were higher than at two remote sites on Lake Superior (Eagle Harbor and Brule River), suggesting that the central Massachusetts background site was affected by local and/or upwind sources of air pollution to a greater extent than the Lake Superior sites (*36*).

Mutagenicity Attributable to Identified Mutagens. Thirty-one of the compounds identified in the annual composites have been tested for mutagenicity in h1A1v2 cells (*27, 28*). Their measured concentrations were multiplied by their specific mutagenicities to calculate the mutagenicity attributed to them (Table 4 and Figure 2). These compounds accounted for 13−22% of the mutagenic potency of the unfractionated extracts (Figure 3). Unsubstituted PAH and a single PAH ketone were among the most important mutagens identified in the samples (Table 4 and Figure 2B). The most important unsubstituted PAH mutagen at each site was benzo[*a*]pyrene (MW 252 amu), which accounted for 2.2−3.3% of the mutagenic potency of the unfractionated extracts. Other PAHs that contributed significantly to the mutagenicity of the samples included cyclopenta[*cd*]pyrene (0.7−2.2%), benzofluoranthenes (the sum of benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene; cumulatively 2.0−2.9%), indeno[1,2,3-*cd*]pyrene (1.3−2.4%), benzo[*ghi*]perylene (0.6−1.7%), 1-methyl-benzo[*a*]pyrene (1.0−1.5%), and dibenzo[*a,e*]pyrene (1.5−1.9%). As a group, the MW 252 amu PAHs accounted for 4−6% of the mutagenic potency of the unfractionated samples, while MW 276−278

TABLE 4. Percentage of Total Mutagenicity Attributable to Known Mutagens

			Rochester (RO)			Quabbin Summit (QB)			Kenmore Square (KS)		
				attributable mutagenicity			attributable mutagenicity			attributable mutagenicity	
	compound[a]	specific potency[b] IMF × 10^6 mg	concn[c] (ng/mg EOC)	IMF × 10^6 mg EOC	%[d]	concn[c] (ng/mg EOC)	IMF × 10^6 mg EOC	%[d]	concn[c] (ng/mg EOC)	IMF × 10^6 mg EOC	%[d]
	Nonpolar Fraction 1 (NP1)										
1	acenaphthylene (AN)	0.08 +	10.9	0.001	0.0	5.0	0.000	0.0	6.3	0.001	0.0
10	1-methylphenanthrene (1-MePh)	0.17 +	7.4	0.001	0.0	3.4	0.001	0.0	32.0	0.005	0.0
	Nonpolar Fraction 2 (NP2)										
35	8-methylfluoranthene (8-MeF)	0.1 +	18.5	0.00	0.0	6.0	0.00	0.0	34.4	0.00	0.0
43	benzo[*c*]phenanthrene (BcPh)	7.3 ++	9.0	0.07	0.1	3.8	0.03	0.0	13.2	0.10	0.1
47	cyclopenta[*cd*]pyrene (CPP)	600 +++	2.2	1.34	2.0	0.7	0.41	0.7	3.5	2.11	2.2
48	benz[*a*]anthracene (BaA)	2 ++	31.6	0.06	0.1	15.2	0.03	0.1	48.5	0.10	0.1
49	chrysene & triphenylene (Chr&Trp)	2 ++	97.6	0.20	0.3	48.3	0.10	0.2	108.7	0.22	0.2
60	benzo[*b*+*j*+*k*]fluoranthene (B[*b*+*j*+*k*]F)	15 ++	140	2.01	3.1	82	1.23	2.0	130	1.95	2.0
62	benzo[*e*]pyrene (BeP)	1 +	68.6	0.07	0.1	38.3	0.04	0.1	69.6	0.07	0.1
63	benzo[*a*]pyrene (BaP)	65 +++	34.1	2.22	3.3	20.1	1.31	2.2	37.0	2.41	2.5
65	1-methylbenzo[*a*]pyrene (1-MeBaP)	99 +++	10.0	0.99	1.5	6.0	0.59	1.0	15.0	1.49	1.5
	Semipolar Fraction (SP)										
69	dibenz[*aj*]anthracene (DBajA)	12 ++	16.7	0.20	0.3	11.6	0.14	0.2	17.6	0.21	0.2
70	indeno[123-*cd*]pyrene (IcdP)	20 ++	81.0	1.62	2.4	39.8	0.80	1.3	86.3	1.73	1.8
71	dibenz[*ah*]anthracene (DBahA)	9 ++	13.6	0.12	0.2	11.2	0.10	0.2	15.5	0.14	0.1
74	benzo[*ghi*]perylene (BghiP)	13 ++	86.6	1.13	1.7	28.4	0.37	0.6	85.5	1.11	1.1
77	N[12-k]F[e]	8 ++	6.5	0.05	0.1	4.5	0.04	0.1	5.1	0.04	0.1
77	N[12-b]F[e]	1.8 +	9.3	0.02	0.0	6.5	0.01	0.0	7.3	0.01	0.0
78	N[23-b]F[e]	8.3 ++	2.0	0.02	0.0	1.2	0.01	0.0	1.5	0.01	0.0
78	DB[bk]F[e]	20 ++	8.9	0.18	0.3	5.6	0.11	0.2	6.6	0.13	0.2
80	N[23-e]P	48 +++	2.8	0.14	0.2	N. D.			4.5	0.21	0.2
82	DB[ae]P	245 +++	5.2	1.29	1.9	4.3	1.05	1.7	5.8	1.43	1.5
83	N[21-a]P[e]	24 ++	3.2	0.08	0.1	2.0	0.05	0.1	3.8	0.09	0.1
83	DB[el]P[e]	6 ++	2.5	0.02	0.0	1.6	0.01	0.0	3.0	0.02	0.0
102	phenalenone (PLK)	0.2 +	100	0.02	0.0	32	0.01	0.0	230	0.05	0.0
89	2+3-nitrofluoranthene (2+3NF)	1.3 +	26	0.03	0.1	14	0.02	0.0	N. D.		
114	7*H*-benz[*de*]anthracen-7-one (BAK)	0.5 +	120	0.06	0.1	90	0.04	0.1	230	0.11	0.1
117	6H-benzo[*cd*]pyren-6-one (BPK)	16 ++	180	2.89	4.3	92	1.47	2.4	220	3.54	3.7
	Polar Fraction (P)[f]										
	total attributed mutagenicity			14.8	22[g]		7.9	13[g]		17.3	18[g]
	mutagenicity of unfractionated sample			68			60			97	
	unattributed mutagenicity of unfractionated sample			53	78		52	87		80	82

[a] Abbreviations: dibenzo[*bk*]fluoranthene (DBbkF), naphtho[1,2-*k*]fluoranthene (N12kF), naphtho[1,2-*b*]fluoranthene (N12bF), naphtho[2,3-*b*]fluoranthene (N23bF), dibenzo[*el*]pyrene (DBelP), naphtho[2,1-*a*]pyrene (N21aP). Benzo[*b*+*j*+*k*]fluoranthene (number 60) are the sum of benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene, which coelute as a triple peak on the HP-5MS column. 2+3-Nitrofluoranthene (number 89) are the sum of 2-nitrofluoranthene and 3-nitrofluoranthene, which coelute on the HP-5MS column. Other abbreviations used in the figures are listed in parentheses. [b] Specific potency is based on mutagenicity data in references *27* and *28*. Amount of mutagenicity is flagged as described in Table 2 (+, ++, +++). 2+3-Nitrofluoranthene (number 89) was assigned the specific potency of 2-nitrofluoranthene (see discussion in text). [c] Concentration in unfractionated extract in ng/mg EOC (from Table 2). N. D. = not detected [d] The fraction of the total mutagenicity of unfractionated extract attributable to the compound listed. [e] Those pairs of MW 302 PAH which coeluted on the GC−MS were assumed to be distributed according to the concentrations reported by Schubert et al. (*35*) for SRM 1649a. [f] Among compounds identified in the Polar fraction, none are known to be h1A1v2 mutagens. [g] If the specific mutagenicity for individual compounds is calculated using steeper slopes in the dose−response plots, the known h1A1v2 mutagens could account for as much as 23−37% of the mutagenic potency of the unfractionated extracts.

amu PAHs accounted for 2−5% and MW 302 amu PAHs accounted for 2−3%. 5-Methylchrysene and dibenzo[*a,l*]-pyrene, two highly mutagenic PAHs, were not detected in any of the samples (limits of detection ≈ 100 pg/mg EOC). Similarly, nitro-PAHs were not found at high levels in any of the samples (limits of detection ∼1−2 ng/mg EOC). Nitro-PAHs are potent mutagens to certain lines of bacteria (*46*) and human MCL-5 cells (*23*) but are in general weakly mutagenic to h1A1v2 cells (28). For purposes of calculating attributed mutagenicity, the coeluting 2- and 3-nitrofluoranthenes (2NF and 3NF) were assigned the specific mutagenicity of 2NF (1.3 IMF × $10^6/\mu$g) because it is generally the more abundant isomer in ambient samples (*47*), and is 3-fold more mutagenic in the h1A1v2 assay than 3NF. Even using the higher specific mutagenicity, the contribution of 2NF was not significant (<0.1% of the total mutagenicity of the samples).

The single most important mutagen at each of the sites was the PAH ketone 6*H*-benzo[*cd*]pyren-6-one, a moderately mutagenic compound, which due to its abundance accounted for 2.4−4.3% of the mutagenicity of the unfractionated extracts. Two other weakly mutagenic PAH ketones—phenalenone and 7*H*-benz[*de*]anthracen-7-one—were present in relative abundance in the three samples, but did not

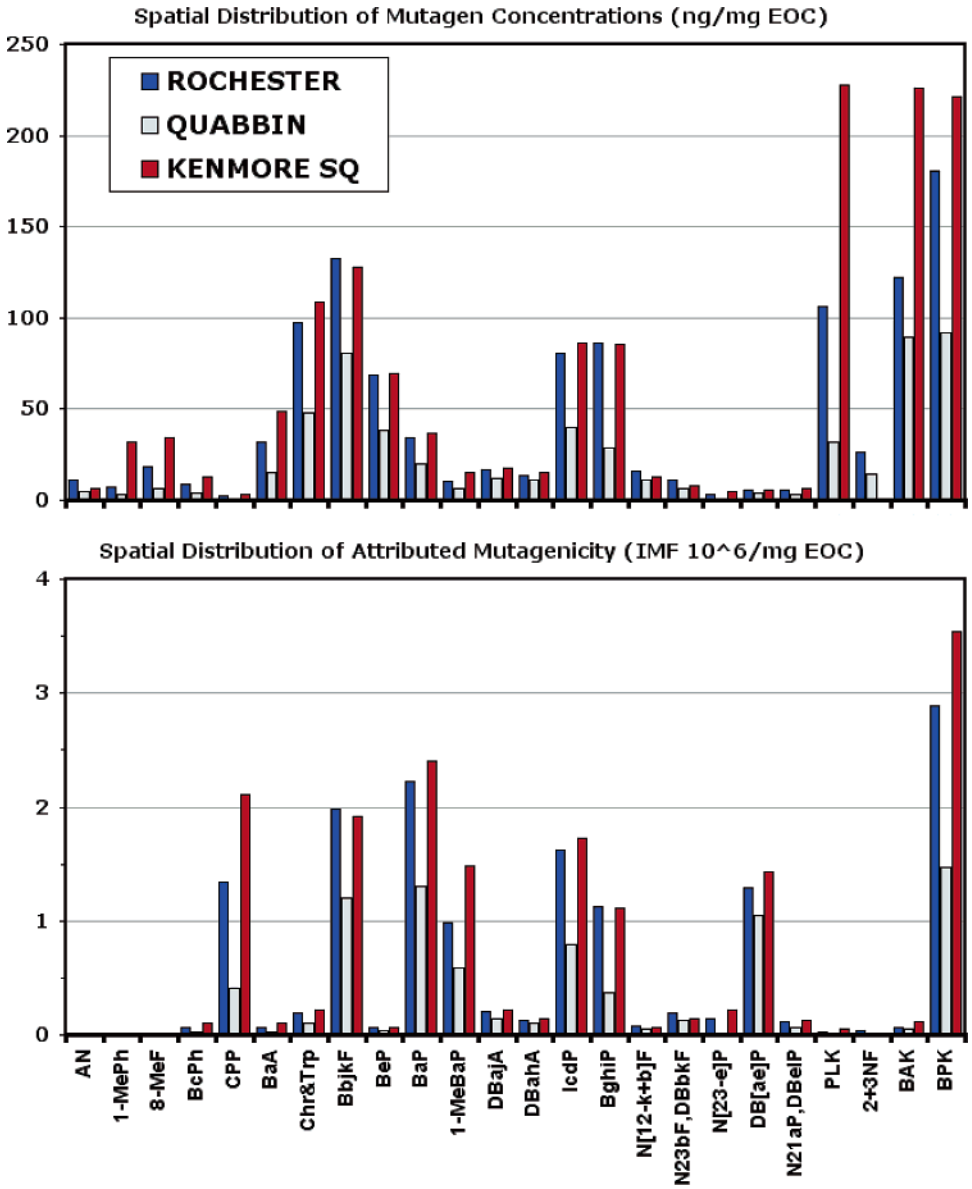

FIGURE 2. Spatial distributions of known mutagens and their attributed mutagenicity at RO, QB, and KS: (A) ppm concentrations, (B) concentration in the sample multiplied by specific mutagenicity. Abbreviations are listed in Table 4.

account for significant amounts of the total mutagenicity of the samples.

The majority of the mutagens were detected in the NP2 and SP fractions, although less than 13% of the mutagenicity of each fraction was attributed to known mutagens (Figure 3). The NP2 and SP fractions were the most mutagenic at KS (representing 28% and 44%, respectively, of the mutagenicity of the sum of the fractions), while at RO the SP fraction was the most mutagenic (68%), followed by the NP2 fraction (17%) and the P fraction (14%). At QB the mutagenicity was evenly distributed among the NP2 (27%), SP (35%), and P (32%) fractions (Table 5). The 12 known mutagens in the NP2 fractions accounted for 6−10% of the mutagenicity of the unfractionated samples (11−13% of the mutagenicity of the NP2 fractions), and the 16 known mutagens in the SP fractions accounted for 7−12% of the mutagenicity of the unfractionated samples (4−10% for the mutagenicity of the SP fractions). In contrast, while the NP1 fractions accounted for 2−10% of the total sample mutagenicity at the three sites, the two known mutagens present in the fraction accounted for <0.01% of the unfractionated sample mutagenicity (0.01−0.03% of the NP1 fraction mutagenicity). No known mutagens were detected in the P fractions, which contained 14−32% of the total mutagenicity of the samples (48−66% of the mutagenicity of the unfractionated sample).

Two important conclusions can be drawn from the results in Tables 2 and 4. First, PAHs and PAH derivatives are important mutagens in $PM_{2.5}$ at the sites sampled in NY and MA. Identical compounds account for the same portion of attributed mutagenicity at all three sites (Figure 4). This is consistent with the results of bioassay-directed chemical analysis studies involving airborne particles from other geographic areas (e.g., *4*−*8, 14, 15*). Second, our results show that >74% of the mutagenicity of the unfractionated samples, >80% of the mutagenicity in the NP2 and SP fractions, and all of the mutagenicity of the P and NP1 fractions is unattributed (Figure 3). This conclusion is consistent with two previous studies that have used h1A1v2 cells to measure mutagenicity in airborne particles. In a study with SRM 1649 (total suspended particulate matter from Washington, DC), 13 PAHs and oxygenated-PAHs accounted for ∼15% of the total mutagenicity of the sample, and the remainder of the mutagenicity was present in fractions that contained semi-polar and polar compounds (*15*). Similarly, in a study of $PM_{2.5}$ from Los Angeles (*14*) it was found that six unsubstituted PAHs, 2-nitrofluoranthene, and 6*H*-benzo[*cd*]pyren-6-one

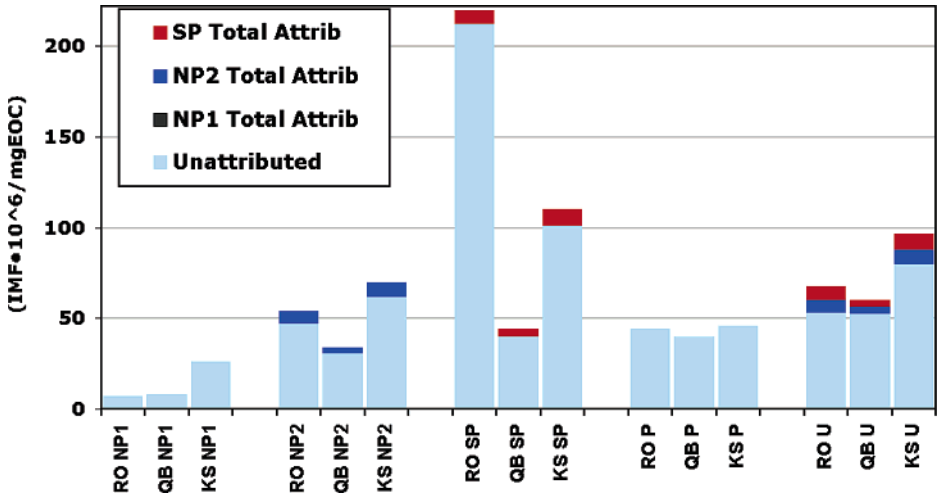

FIGURE 3. Measured mutagenicity of the extracts and their fractions, and the portion attributable to known mutagens. The total height of the bars represents the measured mutagenicity for each extract and fractions thereof (Table 5). The colored areas represent the cumulative amount of mutagenicity attributed to known mutagenic compounds (Table 4). The contribution to mutagenicity of compounds in the NP1 fraction is too small to be visible.

TABLE 5. Mutagenic Potency (IMF × 10^6/mg EOC) of $PM_{2.5}$ from the Northeastern United States: Summary of Previous Studies[a]

	RO	QB	KS
(A) Bimonthly Composites			
JAN–FEB	370	38	58
MAR–APR	190	55	62
MAY–JUN	90	92	29
JUL–AUG	70	60	45
SEP–OCT	180	86	86
NOV–DEC	280	195	171
annual average (±SD)	190 ± 50	80 ± 20	75 ± 20
(B) Annual Composites			
unfractionated	68	60	97
NP1	7 (2%)	8 (6%)	26 (10%)
NP2	54 (17%)	34 (27%)	70 (28%)
SP	220 (68%)	44 (35%)	110 (44%)
P	44 (14%)	40 (32%)	46 (18%)
sum of fractions	325 (100%)	126 (100%)	252 (100%)

[a] (A) Bimonthly composites and their annual averages (*16*). (B) Annual composites and associated fractions (*17*). The percentage of the total mutagenicity (considered equal to the sum of the fractions) represented by each fraction is shown in parentheses.

could account for as much as 19% of the mutagenicity, while the majority of mutagenicity in the sample was attributable to as-yet unidentified compounds in semipolar and polar fractions.

When using our results to evaluate the overall importance of airborne nitro-PAHs as human cell mutagens, it should be noted that other human cell lines have different sensitivities to nitro-PAHs. For example, mono- and dinitro-pyrenes are highly mutagenic to human MCL-5 cells (*23*), which express five cytochrome P450 metabolizing enzymes and microsomal epoxide hydrolase, whereas h1A1v2 cells express just CYP1AI. Thus, it is possible that the importance of nitro-PAHs as airborne mutagens may differ if tested in assays based on other human cell lines.

Spatial Differences in Mutagen Levels. The concentrations of known mutagens (ng/mg EOC) were higher in samples from the two urban sites (KS and RO) compared to the rural site (QB) (Table 4 and Figure 2a). This difference may be attributable to differences in $PM_{2.5}$ sources in rural compared to urban areas, and to reactions (e.g., photodegradation) during atmospheric transport of $PM_{2.5}$ from upwind source areas to rural areas (*48, 49*). In addition, because the amounts of EOC per m^3 of air at KS and RO were higher than at QB, the concentrations of mutagens per m^3 were also higher at KS and RO (Table 2). Comparing the two urban sites (KS and RO), the concentrations (ng/mg EOC) of known mutagens were not significantly different in the NP1 and NP2 fractions, while in the SP fractions, concentrations of mutagenic oxy-PAHs were higher at KS than at RO (Figure 2a).

Previously, we reported that the mutagenic potencies of samples from urban sites (RO and KS) were high relative to QB, the rural background site, especially in the annual composites and the NP2 and SP fractions (Table 5). We also observed that the mutagenic potencies of the SP fractions of the annual composite samples from RO were ~2-fold more mutagenic than those collected at KS and QB. It was expected that these differences would be reflected in the chemical composition of the annual composite samples, and in the attributed mutagenicity. As shown in Figures 2 and 5, however, our expectations were only partially met. The concentrations (ng/mg EOC) of mutagenic compounds in the KS sample were ~2-fold higher than in the QB sample, which could explain why the mutagenicity of the KS sample was high compared to the QB sample. However, there was no significant difference between the KS and RO samples (either in the concentrations of mutagens or in the levels of attributed mutagenicity); thus, the concentrations of known mutagens do not explain the differences in the mutagenicity of these two samples.

The observed differences in mutagenicity may be attributable to different concentrations of as-yet unidentified or unknown mutagens in the KS and RO samples. Many compounds, particularly those in the SP and P fractions, were either not positively identified or not yet tested in h1A1v2 cells; some of these could be important mutagens. However, nearly all of the untested compounds reported in Table 2 showed spatial variations that were inconsistent with the interstate variations observed in the mutagenicity, i.e., RO greater than KS and QB (Figure 5). One candidate is 3-nitrobenzanthrone (3-NB), a potent h1A1v2 cell mutagen that has been identified in $PM_{2.5}$ (*11, 12*). Benzanthrone (7*H*-benz[*de*]anthracen-7-one), presumably the molecule from which 3-NB derives, eluted in the SP fraction. It follows therefore that 3-NB would elute either in this or the P fraction, due to the addition of the polar $-NO_2$ moiety. The KS sample contained nearly twice as much benzanthrone as the RO sample; thus, it is reasonable to hypothesize that there may be a difference in 3-NB levels in the two samples. Other polar

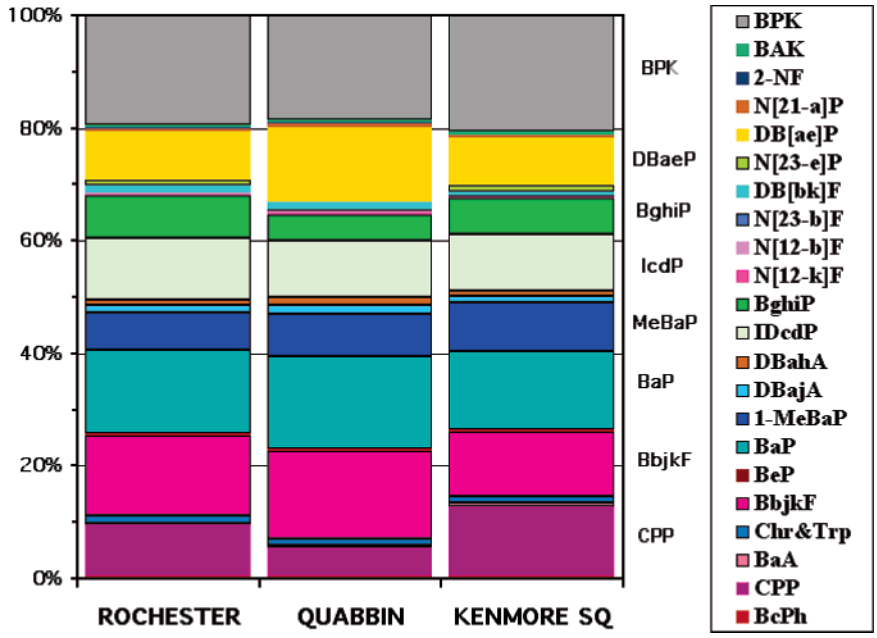

FIGURE 4. Relative contribution of individual human cell mutagens to the total attributed mutagenicity. The attributed mutagenicity of all 31 mutagens (Table 4) are included; the following 8 compounds account for a significant percentage: CPP (cyclopenta[*cd*]pyrene), BbjkF (benzo[*b*]fluoranthene + benzo[*j*]fluoranthene + benzo[*k*]fluoranthene), BaP (benzo[*a*]pyrene), MeBaP (1-methylbenzo[*a*]pyrene), IcdP (indeno[*cd*]pyrene), BghiP (benzo[*ghi*]perylene), DBaeP (dibenzo[*ae*]pyrene), BPK (6*H*-benzo[*cd*]pyren-6-one). The identities and relative importance of the most important human cell mutagens are similar at all three sites.

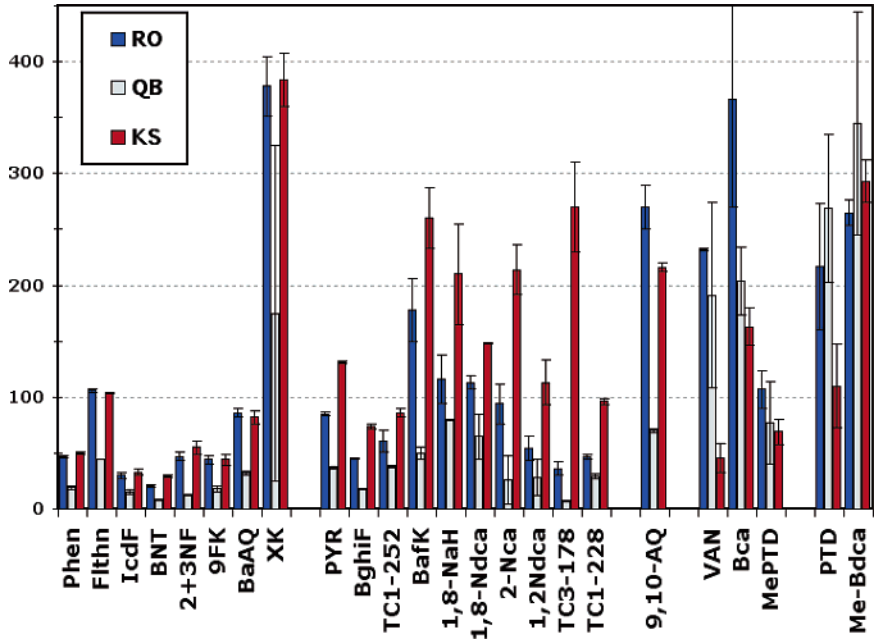

FIGURE 5. Spatial distribution of nonmutagenic aromatic compounds, or those not yet tested in the h1A1v2 assay. Distribution RO ≈ KS > QB includes phenanthrene (Phen), fluoranthene (Flthn), indeno[*cd*]fluoranthene (IcdF), benzonaphthothiophene (BNT), 2+3-nitrofluoranthene (2+3-NF), 9-fluorenone (9FK), benzo[*a*]anthracene-quinone (BaAQ), xanthone (XK). Distribution KS > RO > QB includes pyrene (PYR), benzo[*ghi*]fluoranthene (BghiF), total C1-MW252 PAH (TC1-252), benz[*a*]fluorenone (BafK), 1,8-naphthalic anhydride (1,8-NaH), 1,8-naphthalene-dicarboxylic acid (1,8-Ndca), 2-naphthoic acid (2Nca), 1,2-naphthalene-dicarboxylic acid (1,2Ndca), total C3-MW178 PAH (TC3-178), total C1-MW228 PAH (TC1-228). Distribution RO > KS ≫ QB includes 9,10-anthracenequinone (9,10-AQ). Distribution RO > QB > ~KS includes, vanillin (VAN), benzoic acid (Bca), methylphthalide (MePTD). Distribution QB > RO > ~KS includes phthalide (PTD), methyl-benzene dicarboxylic acid (Me-Bdca). Error bars indicate the standard deviation of three measurements (Table 2).

compounds that are candidates for mutagenicity testing include hydroxy-PAHs and hydroxynitro-PAHs, some of which are potent mutagens to bacteria (*50*). Benzoic acid and methylphthalide, and to a lesser extent vanillin, exhibited spatial distributions that were similar to the interstate variability of the mutagenic potency of the bimonthly composites; therefore, these compounds should also be considered for mutagenicity testing in h1A1v2 cells.

Sources. We used our results to help identify possible sources of chemicals to the $PM_{2.5}$ samples. Other studies have used ratios of alkylated PAH homologues to distinguish pyrogenic (e.g., combustion emissions) and petrogenic (e.g., unburned fuel) PAH sources (*51*−*53*). Pyrogenic emissions are characterized by relatively high levels of more stable unalkylated PAHs and relatively low levels of alkylated homologues compared to petrogenic emissions (*52*) (Figure

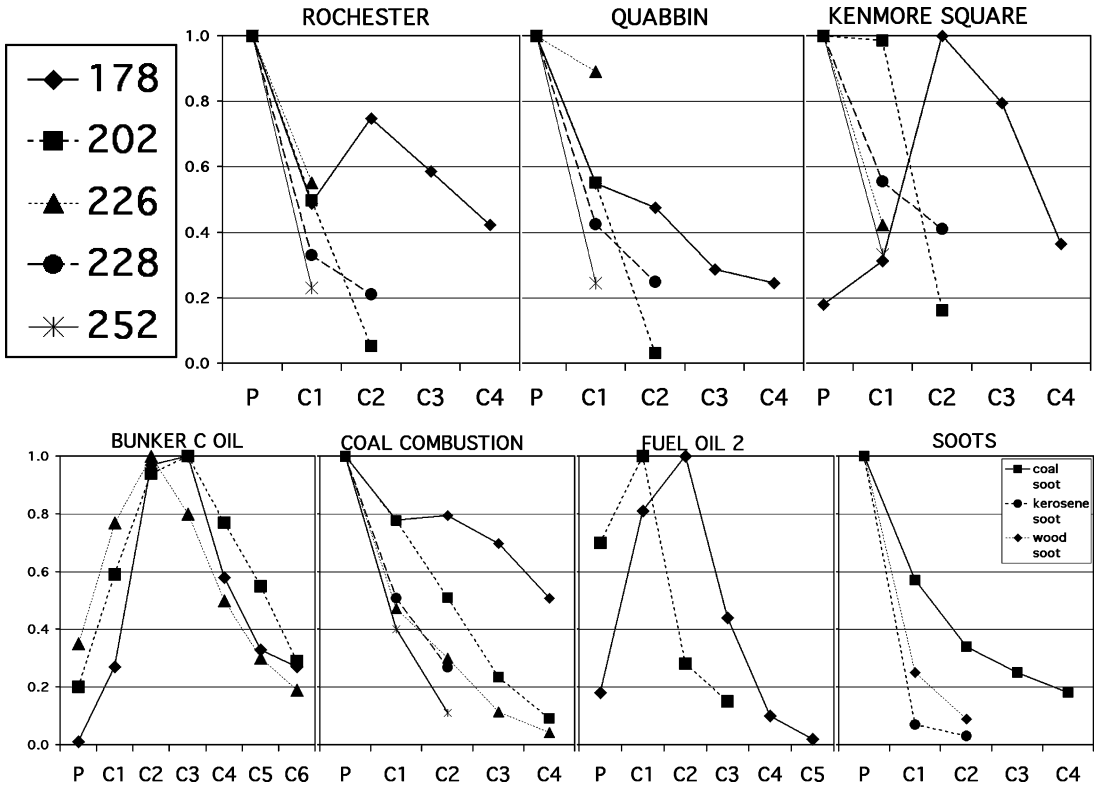

FIGURE 6. PAH alkyl homologue ratios for ambient $PM_{2.5}$ and selected emission particles. The legend refers to the molecular weight (MW) of the unalkylated PAH; all PAHs and alkyl PAHs of the same MW were summed. "P" refers to the unalkylated parent compound; "C*n*" refers to the number of alkyl carbons. Data for the Rochester, Quabbin, and Kenmore Square samples are taken from Table 2. Representative plots for petrogenic (Bunker C Oil and No. 2 distillate fuel oil) and pyrogenic (coal combustion particles) materials are taken from Youngblood and Blumer (*51*). Composite alkyl homologue ratios for combustion products of coal, kerosene, and wood (soots) are taken from Lee et al. (*52*).

6). The RO, KS, and QB samples contained relatively high levels of unalkylated PAH and relatively low levels of alkylated PAH homologues (Figure 6), suggesting that emissions from pyrogenic sources were significant contributors to EOC in $PM_{2.5}$ at these sites compared to petrogenic sources. At KS, higher ratios of lower-MW alkyl-PAHs were observed compared to the other sites, possibly indicating contributions from petrogenic sources, such as unburned fuel from local traffic. Another indicator of fresh emissions, the highly reactive cyclopenta[*cd*]pyrene, was measured at relatively high concentrations at KS. Similarly, the higher abundances of 2-nitrofluorene and 9-nitroanthracene at KS than at RO and QB suggest a higher contribution of direct diesel emissions at KS (*47*). In addition, 2-nitrofluoranthene, usually more abundant in particles that have undergone atmospheric reactions (*47*), was higher in $PM_{2.5}$ from RO and QB than KS, which is consistent with RO and QB being sites where more weathered material is collected compared to KS.

Significance. The results of this study add to our knowledge of the distribution of human cell mutagens in $PM_{2.5}$ in the United States. The similarities among the RO, KS, QB, and Los Angeles $PM_{2.5}$ (*14*), and the Washington, DC TSP (*15*) indicate that known human cell mutagens are widely distributed in rural and urban areas throughout the United States. Interestingly, known human cell mutagens accounted for similar percentages (13−22%) of the unfractionated sample mutagenicity at these five sites. Furthermore, the finding that unsubstituted-PAHs and oxy-PAHs are important mutagens in the RO, KS, and QB samples suggests that combustion emissions, many of which contain high levels of PAHs (*54*−*56*), are significant sources of mutagens in $PM_{2.5}$, and that their distribution is similar across the entire region. This implies either that the same sources affect all of the sites similarly, suggesting long-range transport, or that similar emission sources with more local effects are widely distributed. While our results are generally similar to those of the Los Angeles study, there are some important differences. For example, the greater importance of nitro-PAH mutagens in Los Angeles compared to the three northeast U.S. sites may reflect regional differences in the conditions that give rise to nitro-PAHs—e.g., atmospheric transformation of unsubstituted PAHs to nitro-PAHs (*9, 57*−*60*).

A second significant finding of our study is that only ~20−30% of the human cell mutagenicity of the $PM_{2.5}$ samples could be attributed to known mutagens, and of this most was attributable to unsubstituted PAHs, alkyl-PAHs, and PAH ketones present in nonpolar and semipolar fractions. This finding is consistent with the Los Angeles and Washington, DC studies (*14, 15*). In addition, regional differences measured in the mutagenicity of the SP fractions cannot be explained by the mutagenicity attributed to mutagens in the SP fraction or the spatial distribution of other, nonmutagenic compounds. Interestingly, significant (95% CL) spatial differences in mutagenicity were only observed in the bimonthly composites and fractionated extracts of the annual composites. These observations support the hypothesis that mutagens in samples may be subject to synergistic reactions with as-yet unidentified organic compounds (*61*−*63*). This hypothesis could be evaluated by testing known mutagens and mixtures of known mutagens for mutagenicity in the presence and absence of nonmutagenic fractions so as to determine whether synergistic effects, such as enhancement or inhibition, may reasonably be expected when testing whole samples for mutagenicity.

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