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Human Lymphoblast Mutagens in Urban Airborne Particles

JOHN L. DURANT,*,† ARTHUR L. LAFLEUR,‡ ELAINE F. PLUMMER,‡ KOLI TAGHIZADEH,‡ WILLIAM F. BUSBY JR.,‡ AND WILLIAM G. THILLY‡,§

Department of Civil and Environmental Engineering, Tufts University, Medford, Massachusetts 02155, Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Division of Toxicology, Whitaker College of Health Sciences and Technology, Cambridge, Massachusetts 02139

While it is known that urban airborne particles typically contain trace levels of bacterial mutagens and rodent carcinogens, little work has been done to identify chemicals in such particles that can genetically alter human cells. In this paper, we describe the analysis of an organic extract of a Washington, DC, airborne particle sample (SRM 1649) for human cell mutagens. Due to the chemical complexity of the extract, a bioassay-directed fractionation method was used to separate mutagenic constituents into chemically simplified fractions. Mutagenicity testing was done using the h1A1v2 cell line, a line of human B-lymphoblastoid cells that have been engineered to overexpress the human cytochrome P4501A1. Chemical analysis of mutagenic fractions was accomplished using GC−MS and HPLC−UV techniques. Our results indicate that ∼20% of the total mutagenicity the extract was accounted for in two fourth-order fractions that contained ∼3% of the total extract mass. These fractions were composed largely of polycyclic aromatic hydrocarbons (PAH). A total of 13 PAH were identified that accounted for ∼15% of the mutagenicity of the extract. Of these, the most important mutagens were cyclopenta[*cd*]pyrene, benzo[*a*]-pyrene, and benzo[*b*]fluoranthene, accounting for ∼7, ∼4, and ∼2%, respectively, of the extract mutagenicity. Naphtho-[2,1-*a*]pyrene (N[2,1-*a*]P) and naphtho[2,3-*a*]pyrene (N[2,3-*a*]P), two previously unknown potent human lymphoblast mutagens, were also identified in the sample. N[2,1-*a*]P accounted for ∼3% of the extract mutagenicity; N[2,3-*a*]P, which was present at relatively low levels, accounted for <1% of the extract mutagenicity. The remainder of the mutagenicity was found in fractions that contained more polar compounds. One of these polar fractions contained many different classes of oxygenated polycyclic aromatic compounds (oxy-PAH) including ketones, quinones, coumarins, and carboxylic acid anhydrides; however, of the mutagenic oxy-PAH identified, only the ketone 6*H*-benzo-[*cd*]pyren-6-one (∼0.5%) was found to account for a significant portion of the total mutagenicity of the extract. Nitro-PAH, many of which are potent bacterial mutagens, did not contribute significantly to the mutagenicity of this sample because they were present at low concentrations and because they are not particularly mutagenic in h1A1v2 cells.

* Corresponding author e-mail: jdurant@tufts.edu; fax: 617-627-3994; phone: 617-628-5000, ext. 5489.

† Tufts University.

‡ Massachusetts Institute of Technology.

§ Whitaker College of Health Sciences and Technology.

Introduction

It has long been known that chemicals associated with urban airborne particles can cause cancer. Campbell (*1, 2*) was one of the first to report this when he demonstrated in the 1930s that mice developed lung tumors after inhaling automobile exhaust and dust from tarred roads. Since then, several investigators have shown that organic solvent extracts of urban airborne particles can cause cancer in laboratory animals (*3*−*9*). Although such findings suggest that exposure to urban airborne particles may cause cancer— particularly lung cancer—in humans, proof of causality has not been established. This is due in no small measure to the difficulty of establishing the role that environmental pollutants play in causing cancer in general, but in the specific case of linking air pollution to lung cancer, the problem is greatly compounded by the effects of smoking. Smoking is by far the dominant cause of lung cancer, accounting for nearly 90% of all lung cancer cases in the United States (*10*). Nonetheless, it has been reported that lung cancer rates are as much as 1.5−2-fold higher in urban areas than in rural areas (*11*−*14*); for nonsmokers in particular, lung cancer rates are generally 1.05−1.4-fold higher in urban areas than in rural areas (*15*−*17*). Such reports have strengthened the belief that pollutants in urban air are contributing to the incidence of lung cancer.

In the last 20 years, numerous papers have reported the occurrence of genotoxic chemicals (i.e., chemicals that can alter DNA) in urban air samples (see reviews in refs *18*−*20*). This productivity has been fueled in part by the development of short-term mutation assays, most notably the Ames assay, which is based on the bacterium *Salmonella typhimurium*. Because mutations are required for the formation of cancer cells, mutation assays have been employed as screening tools for selecting candidate compounds for carcinogenicity testing. By coupling the use of such assays with chemical analysis techniques, it has been shown that urban air—especially in industrialized areas—typically contains dozens of mutagenic chemicals (*18, 19*). The most commonly reported are polycyclic aromatic hydrocarbons (PAH) (*18, 19, 21, 22*) and nitrogen-containing polycyclic aromatic compounds such as nitro-PAH (*23*), hydroxynitro-PAH (*24*), and nitro-PAH lactones (*25*).

To date, little work has been done to identify chemicals in urban air that can mutate human cells. Part of the reason for this is that until recently human cell lines competent for xenobiotic metabolism were not available for routine mutagenicity testing. In this paper, we describe the analysis of an organic extract of an urban airborne particle sample for chemicals that can mutate human h1A1v2 cells. h1A1v2 cells are human B-lymphoblastoid cells that constitutively overexpress the oxidative enzyme cytochrome P4501A1 (*26*). Due to the chemical complexity of the extract, we used a bioassay-directed fractionation method to separate mutagenic chemicals into fractions that were more amenable to instrumental analysis. Our goal was to identify the *most important* human lymphoblast mutagens in the sample (i.e., those chemicals that, due to the combination of their mutagenic potency and abundance, accounted for the largest fraction of the sample mutagenicity). The contribution of individual compounds

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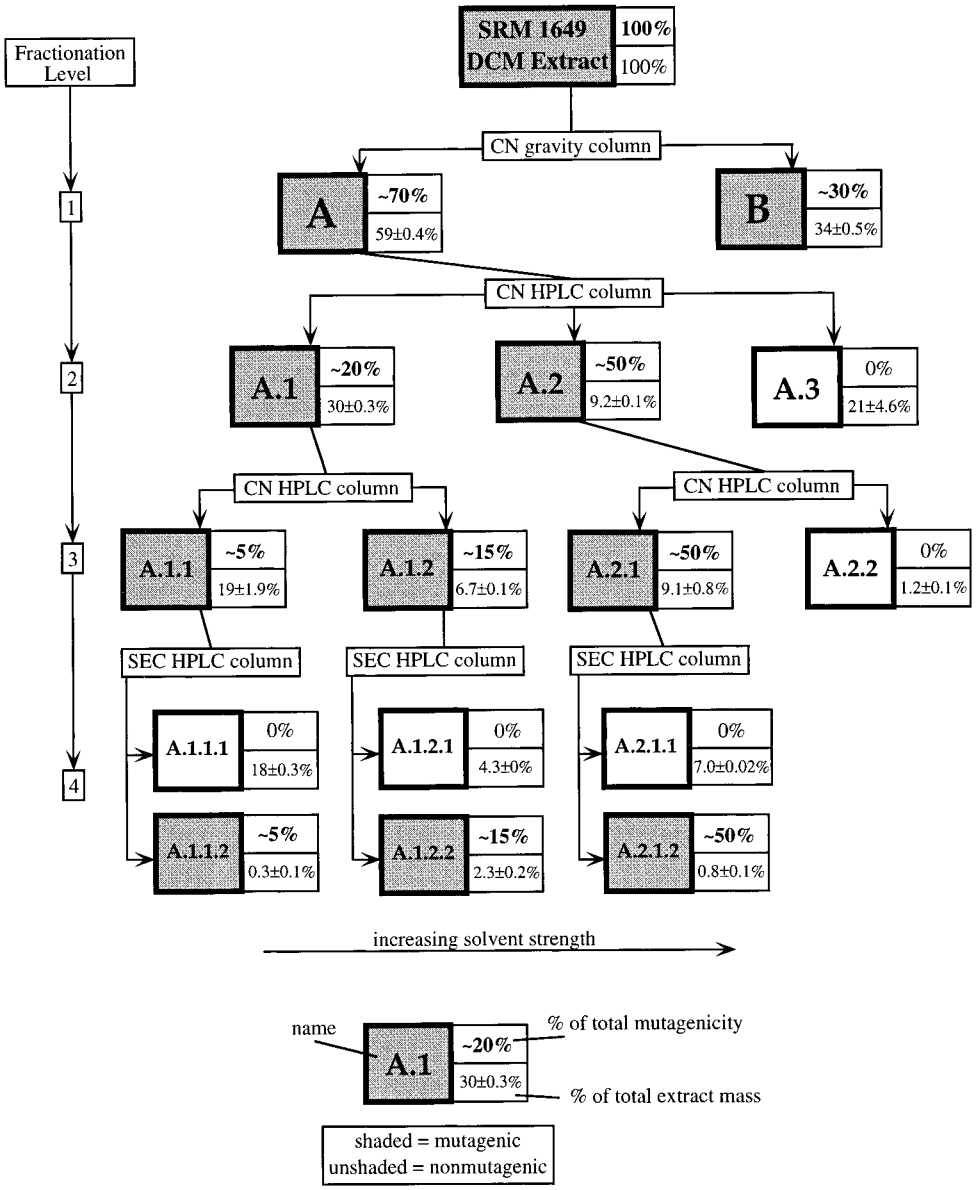

FIGURE 1. Distribution of human lymphoblast mutagenicity in fractions of an organic extract of a Washington, DC, airborne particle sample (SRM 1649).

to the total mutagenicity of the sample was calculated using a mutagenicity database (n = 80) that has been reported elsewhere (*27, 28*).

Methods and Materials

Sample Preparation. In order to have sufficient amounts of airborne particulate material for both chemical and biological characterization, a sample of Standard Reference Mixture 1649 (SRM 1649) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD). SRM 1649 was collected in Washington, DC, over a ~12-month period in the late 1970s using a baghouse sampler equipped with canvas bags (*29*); this sample has been analyzed previously for mutagenicity in bacteria (*29*−*33*) and organic constituents (*33*−*38*). Subsamples of SRM 1649 were weighed out in 9−10 g amounts, transferred to precleaned glass fiber thimbles, and extracted for 24 h in a Soxhlet extractor with 500 mL of HPLC-grade dichloromethane (CH_2Cl_2). Following extraction of the particles, the extracts were concentrated by vacuum centrifuge to ~40 mL and then filtered through Gelman Acrodisc 0.2 μm Teflon filters (American Bioanalytical; Natick, MA). The volume of the extracts was then reduced to 5−10 mL by evaporation under a gentle stream of N_2 gas. The weight of dissolved material in the resulting concentrate was measured by a microscale evaporation method described previously (*39*). Prior to mutagenicity testing, extract aliquots were exchanged into dimethyl sulfoxide (DMSO).

Fractionation. The extract was fractionated using the four-level bioassay-directed fractionation method shown in Figure 1. In the first fractionation step, disposable precolumns were used to remove sample constituents that could bind irreversibly to HPLC columns. The 20-mg aliquots of the CH_2Cl_2 extract were loaded onto precleaned and conditioned 1-g cyanopropyl (CN) bonded-phase sorbent columns (J&W Scientific; Folsom, CA) and then eluted with 25 mL of CH_2Cl_2 and 20 mL of CH_3OH (in that order) to separate broad classes of nonpolar (fraction A) and polar compounds (fraction B), respectively. CN was chosen as the stationary phase because it has been shown to preserve mutagenicity in complex mixtures during fractionation (*40*).

In the second fractionation step, the A fraction was fractionated on a CN preparatory HPLC column into three second-order fractions: A.1, A.2, and A.3. The solvent

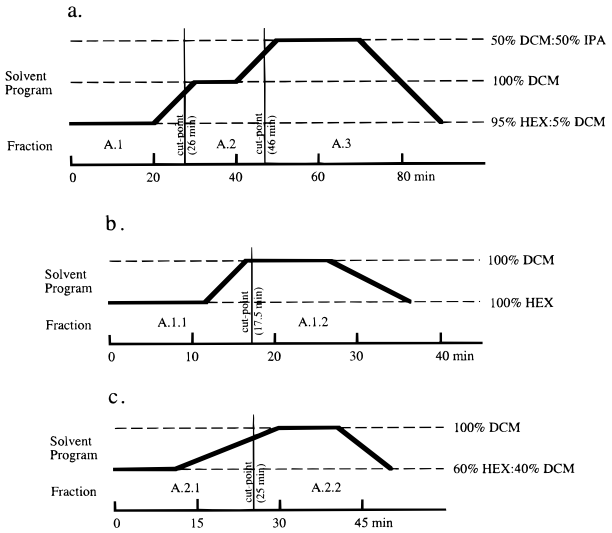

FIGURE 2. Normal-phase chromatography fractionation schemes for the (a) A fraction, (b) A.1 fraction, and (c) A.2 fraction. All three fractions were fractionated on the same CN preparatory column.

program used and the cutpoints for the fractions are shown in Figure 2. The liquid chromatographic system consisted of a Varian (Palo Alto, CA) model 5060 ternary gradient pump coupled to a Hewlett-Packard (Palo Alto, CA) model 8450A diode-array spectrophotometric detector. The spectrophotometer was fitted with a quartz flow cell having a circular aperture of 2.0 mm and a path length of 1.0 cm. The CN column was 25 cm long, 10 mm in diameter, and packed with 10 μm of CN material (Alltech; Deerfield, IL). The flow rate was 4.0 mL/min. Sample injection was performed using a Rheodyne (Cotati, CA) injector with a 500-mL loop.

At the third fractionation level, the A.1 and A.2 fractions were fractionated, again using CN as the stationary phase. The solvent programs that were used and the cutpoints for the fractions are shown in Figure 2. The column and the HPLC system were the same as those described for the second fractionation step. A flow rate of 4 mL/min was maintained in both programs. Fraction A.1 was exchanged into cyclohexane prior to fractionation.

In the fourth fractionation step, size-exclusion chromatography (SEC) was used to fractionate the A.1.1, A.1.2, and A.2.1 fractions. Jordi-Gel poly(divinyl benzene) material (Jordi Associates, Inc.; Bellingham, MA) was used as the stationary phase; CH_2Cl_2 was used as the elution solvent. This combination has been shown to effectively separate broad classes of planar polyaromatic compounds and aliphatic compounds in chemically complex environmental samples (*41*, *42*). The SEC column was 50 cm long, 1.0 cm in diameter, and packed with 500 Å of Jordi-Gel poly(divinyl benzene) material. The liquid chromatographic system was the same as that used in the second and third fractionation steps. CH_2Cl_2 was run at 1.5 mL/min. The retention times of 4*H*-cyclopenta[*def*]phenanthrene and 5,12-naphthacenequinone were used as the cutpoints for fractionating the A.1.1 and A.2.1 fractions, respectively; the earliest elution time of compounds in SRM 1587 (nitro-PAH standard reference mixture) was taken as the cutpoint for fractionating the A.1.2 fraction.

Human Cell Mutation Assay. Mutagenicity was measured at the thymidine kinase (*tk*) locus in h1A1v2 cells using a mutation assay protocol described previously (*43*). Samples and standards dissolved in DMSO were tested at 3−5 doses in duplicate 12-mL cultures along with positive controls (1 μg/mL of benzo[*a*]pyrene in duplicate cultures) and negative controls (60 μL of DMSO in quadruplicate cultures). Each culture initially contained 1.8×10^6 cells, which were allowed to grow exponentially for 72 h. Treatment was terminated by centrifuging the cells and resuspending them in 30 mL of fresh medium. One day later, cells were counted, and fresh medium was again added to bring the cell concentration to 2×10^5 cells/mL. After an additional 2 days of growth to allow for the phenotypic expression of mutations, cells were plated onto 96-well microtiter plates in the presence (mutagenicity) and absence (plating efficiency) of the selective agent trifluorothymidine. In the mutagenicity plates (n = 3), 20 000 cells were added per well; in the plating efficiency plates (n = 2), two cells were added per well. The plates were then incubated for 13 days and scored for the presence of a colony in each well. The CH_2Cl_2 extract and each fraction were tested in at least two independent assays.

The plating efficiency and mutant fraction (i.e., the ratio of surviving mutant colonies to total surviving colonies after plating) were calculated for each culture using methods described by Furth et al. (*44*). The toxicity of each sample was determined as the ratio of surviving treated cells to surviving negative control cells measured from the beginning of treatment until plating. The outcome of an assay was considered to be positive if the responses (i.e., the mean mutant fractions) increased with dose and if the response at one or more doses exceeded both the 95% upper confidence limit of the concurrent negative controls (calculated using Dunnett's *t*-test) and the 99% upper confidence limit of the historical negative controls (*45*).

The percentage of the mutagenicity in each fraction was calculated in the following way. First, the results from replicate mutagenicity tests were pooled. Next, the minimum mutagenic concentration (MMC) (i.e., the dose at which the dose−response curve intersected the larger of the 95% upper confidence limit of the concurrent controls for the pooled results or the 99% upper confidence limit of the historical negative controls) was calculated. The relative mutagenicity of each fraction with respect to its sibling fraction(s) was calculated by comparing MMCs. The percentage of the mutagenicity in individual fractions was then determined by comparing the relative mutagenicities of the sibling fractions to the percentage of the total mutagenicity attributable to the parent fraction. Sibling fractions were tested on the same day to control for any day-to-day variation in the assay.

The percentage of the total mutagenicity accounted for by each mutagenic compound (%Mut$_i$) was calculated as

$$\%\mathrm{Mut}_i = \left(\frac{\mathrm{C}_i \cdot \mathrm{IMF}_i \cdot \mathrm{MMC}_\mathrm{E}}{\mathrm{MMC}_i \cdot \mathrm{IMF}_\mathrm{E}}\right) \times 100$$

where C_i is the mass of chemical i per gram of particulate material; MMC_E and MMC_i are the minimum mutagenic concentrations of the extract and chemical i, respectively; IMF_E and IMF_i are the induced mutant fraction at the MMC of the extract and chemical i, respectively.

Chemical Analysis. The principal method used to analyze mutagenic fractions was gas chromatography with mass selective detection (GC−MS). The GC−MS system consisted of a Hewlett-Packard (HP) 5890II GC and a HP5972 MSD. Data acquisition and analysis were carried out with HP MS ChemStation software. The GC was equipped with a 30 m HP-5 5% methyl-phenyl FSOT column that had an inside diameter of 0.25 mm and a 0.25 μm film thickness. The GC oven was programmed as follows: (i) after injection hold for 1.5 min at 50 °C, (ii) ramp to 310 °C at 8 °C/min, and (iii) hold for 10 min at 310 °C. The flow of the carrier gas (He) was maintained at 1 mL/min; the transfer line temperature was maintained at 280 °C. Samples were injected in 1.0−1.5 μL volumes into a split/splitless injector operated in the splitless mode. The mass range scanned was 50−450 amu in the

electron impact mode (70 ev); the scanning rate was 1.8 s^{-1}. Quantification was done by comparing peak areas of identified compounds with those of known amounts of external standards. Three-point calibration curves were developed with authentic standards for 26 compounds; 18 compounds were quantified using calibration curves from isomers or structurally related compounds (see Table 4). Fractions and standards were injected in triplicate.

To facilitate the analysis of $C_{24}H_{14}$ (6-ring) PAH, a fresh 8.0-g sample of SRM 1649 was prepared. The sample was extracted in CH_2Cl_2 in a Soxhlet extractor for 24 h, concentrated, and filtered as described previously. The extract was then exchanged into toluene and fractionated on an amino column (50 cm × 10 mm × 10 μm average particle diameter; Alltech; Deerfield, IL) based on a method developed by Wise et al. (*36*). The fraction enriched in $C_{24}H_{14}$ PAH was analyzed by reversed-phase liquid chromatographic separation and UV−visible light spectrophotometric detection (LC−UV). The LC−UV system consisted of a HP model 1090 ternary gradient pump coupled to a HP model 1040, 190−600 nm wavelength diode-array detector. Data acquisition and analysis were performed on an HP model 7994 analytic workstation with HP 79995A and 79996A HPLC operating software. The column was a Vydac (Hesperia, CA) 201TP54 polymeric-C_{18} analytical column (25 cm × 4.6 mm × 5.0 μm average particle diameter); the mobile phase was run as a gradient starting from 40:60 CH_3CN/H_2O, ramping to 100% CH_3CN at 40 min, and then to 100% CH_2Cl_2 at 80 min. The flow rate was set at 1.5 mL/min. The fraction was exchanged into DMSO and analyzed by injecting 20 μL aliquots in triplicate. Compounds were identified by comparing retention times and the observed UV spectra with those of authentic standards. Individual $C_{24}H_{14}$ PAH were quantified by comparing their mean peak areas with the response factor for naphtho[2,1-*a*]pyrene, which was determined by injecting three dilutions in triplicate.

Nitro-PAH were analyzed by chemiluminescense-based nitrogen-selective gas chromatography. The instrument consisted of a Hewlett-Packard model 5890 gas chromatograph equipped with a cold on-column injector. The column was a 30-m (5% phenyl)-methyl-silicone fused silica capillary column (J&W Scientific; Rancho Cordova, CA) having an i.d. of 0.53 mm. The GC was coupled to a model 610 TEA analyzer (Thermedics, Inc.; Woburn, MA). Data acquisition and processing were performed using Hewlett-Packard 3365A Chemstation software running on a Vectra PC. The GC column exited through a heated interface (310 °C) normally used for GC−MS coupling and was inserted directly into the TEA pyrolyzer region. The pyrolyzer consisted of a 75 cm length of alumina tubing with an o.d. of 6.0 mm and an i.d. of 2.0 mm. A 50 cm length of 1.0 mm diameter Pt wire was inserted into the pyrolyzer region of the tube to serve as a catalyst. The pyrolyzer was maintained at 950 °C. A complete description of the instrument and its application to the detection of nitroarenes has been previously reported (*46*−*50*).

Chemicals. The benzofluorenone isomers and 7*H*-dibenz[*de*,*j*]anthracen-7-one were obtained from Risø National Laboratories (Roskilde, Denmark); 4*H*-cyclopenta-[*def*]phenanthren-4-one, 6*H*-benzo[*cd*]pyren-6-one, cyclopenta[*cd*]-3(4*H*)-one, anthanthrenequinone, and several $C_{24}H_{14}$ PAH were acquired from the PAH Research Institute (Greifenberg, Germany); 5,5′-dichloro-2,2′-dihydroxybenzophenone (oxy-dichlorophen) was synthesized by Oryza Chemical Co. (Newburyport, MA); naphtho[8,1,2-*abc*]coronene was a gift from Dr. John Fetzer (Chevron Corp.); the remaining analytical standards were purchased from different commercial vendors. The solvents CH_2Cl_2, CH_3OH, CH_3CN, hexane, and 2-propanol, were all high-purity, HPLC-grade and purchased from either EM Science (Gibbstown, NJ) or J. T. Baker (Phillipsburg, NJ). The DMSO was purchased from EM Science.

TABLE 1. Elution Order of Polycyclic Aromatic Compounds on a CN HPLC Preparatory Column

compound	vol_e[a]	solvents[b]	fraction[c]
dibenzothiophene	21.6	95:05 HEX:DCM	A.1
dibenzofuran	24.8	95:05 HEX:DCM	A.1
pyrene	30.0	95:05 HEX:DCM	A.1
dibenz[*a*,*h*]anthracene	47.6	95:05 HEX:DCM	A.1
1-nitronaphthalene	50.0	95:05 HEX:DCM	A.1
9-nitroanthracene	52.3	95:05 HEX:DCM	A.1
1-nitropyrene	70.0	95:05 HEX:DCM	A.1
1-cyanonaphthalene	93.6	63:37 HEX:DCM	A.1
1-pyrenecarboxaldehyde	107	31:69 HEX:DCM	A.2
1,3-dinitropyrene	109	26:74 HEX:DCM	A.2
carbazole	114	14:86 HEX:DCM	A.2
1,8-dinitronaphthalene	118	05:95 HEX:DCM	A.2
2-aminonaphthalene	125	100% DCM	A.2
phenalenone	126	100% DCM	A.2
1-hydroxypyrene	183	79:29 DCM:IPA	A.2
2,3-dihydroxynaphthalene	184	70:30 DCM:IPA	A.2
2-naphthoic acid	246	50:50 DCM:IPA	A.3

[a] Vol_e is the elution volume. Pumping rate was 4 mL/min. [b] HEX is hexane; DCM is dichloromethane (CH_2Cl_2); IPA is 2-propanol. [c] See Figure 2a for solvent program.

TABLE 2. Recoveries of PAH from Three LC Columns[a]

	HPLC column		
compound	CN-gravity[b] (%)	CN−HPLC[c] (%)	PDVB−SEC[d] (%)
acenaphthylene	91 ± 4	77 ± 2	74 ± 6
acenaphthene	90 ± 4	80 ± 2	78 ± 8
fluorene	93 ± 4	88 ± 2	84 ± 6
phenanthrene	95 ± 4	90 ± 2	94 ± 7
fluoranthene	95 ± 4	97 ± 1	85 ± 7
pyrene	110 ± 8	100 ± 2	90 ± 5
benz[*a*]anthracene	93 ± 4	98 ± 2	90 ± 6
chrysene	95 ± 4	96 ± 2	92 ± 5
benzo[*b*]fluoranthene	97 ± 4	100 ± 2	86 ± 6
benzo[*k*]fluoranthene	96 ± 4	110 ± 2	88 ± 6
benzo[*a*]pyrene	96 ± 4	100 ± 2	84 ± 6
indeno[1,2,3-*cd*]pyrene	95 ± 4	100 ± 3	88 ± 5
dibenz[*a*,*h*]anthracene	95 ± 3	100 ± 4	82 ± 6
benzo[*ghi*]perylene	93 ± 3	97 ± 3	86 ± 6

[a] Recoveries were measured by loading microgram amounts of PAH onto the LC columns, eluting with organic solvents, and quantifying the amount of each PAH recovered in the eluate. Experiments were performed twice on each column; results are presented as the mean recovery ± SD; $n = 6$ (three replicate analyses per experiment). [b] 1-g cyanopropyl gravity column; elution solvents = 25 mL of CH_2Cl_2) and 20 mL of CH_3OH. [c] See Figure 2a for solvent program. [d] PVDB-SEC is poly(divinyl benzene) size-exclusion column; CH_2Cl_2 was run as the mobile phase.

Results

Fractionation Method Development. The elution order of selected polycyclic aromatic compounds run individually at the second fractionation level is shown in Table 1. The compounds were chosen to represent the broad range of sizes and polarities of aromatic compounds present in urban airborne particulate matter. PAH and mononitro-PAH eluted in the A.1 fraction; semipolar compounds (e.g., dinitro-PAH and PAH ketones) eluted in the A.2 fraction; and naphthoic acid, the most polar compound run on the column, eluted in the A.3 fraction. A mixture of 14 PAH was run on each of the LC columns to estimate recoveries during sample fractionation. As shown in Table 2, the CN column recoveries for most compounds were ≥90%, while the recoveries from the size-exclusion column were somewhat lower (≥82%).

TABLE 3. Mutagenicity of SRM 1649 Extract Fractions in Human Lymphoblast Cells

sample[a]	concn[b] (μg equiv/mL)	mutant fraction[c]	relative survival[d]	MMC[e]
CH_2Cl_2 extract	7.3−600	25 ± 8−48 ± 14	0.90−0.49	180
fraction A	29−2400	29 ± 10−54 ± 5	0.87−0.26	750
fraction B	29−2400	22 ± 1−48 ± 10	1.0−0.42	1800
fraction A.1	7.3−600	25 ± 3−52 ± 11	0.96−0.85	340
fraction A.2	7.3−600	28 ± 8−57 ± 11	0.98−0.85	130
fraction A.3	7.3−2400	24 ± 2−33 ± 4	0.95−0.30	−
fraction A.1.1	7.3−6000	20 ± 0.4−53 ± 14	0.94−0.80	3500
fraction A.1.2	22−6000	20 ± 7−51 ± 9	0.97−0.63	1100
fraction A.2.1	7.3−6000	26 ± 7−67 ± 4	0.89−0.62	1100
fraction A.2.2	7.3−6000	24 ± 0.3−24 ± 2	1.1−0.90	−
fraction A.1.1.1	15−24000	23 ± 2−22 ± 9	1.1−0.95	−
fraction A.1.1.2	15−24000	18 ± 2−44 ± 21	1.1−0.90	6000
fraction A.1.2.1	200−12000	30 ± 15−28 ± 6	0.97−1.1	−
fraction A.1.2.2	200−12000	39 ± 11−110 ± 62	0.99−0.32	4500
fraction A.2.1.1	200−12000	26 ± 6−33 ± 10	0.81−0.78	−
fraction A.2.1.2	200−12000	23 ± 3−61 ± 11	1.0−0.67	1900

[a] See Figure 1 for fractionation scheme. [b] Range of concentrations tested for mutagenicity. Concentrations are expressed as microgram of equivalent unextracted particle mass per mL of cell culture. [c] Mean mutant fractions per 10^6 colony forming cells (± standard deviation) corresponding to the concentrations listed in the second column. The mean response of the negative controls was 23 ± 7.4 (n = 130); the mean response of the positive controls, 1 μg of B[*a*]P per mL of cell culture, was 210 ± 80 (n = 130). Complete mutagenicity test results for the extract and the fractions at all doses tested are available in the supporting material. [d] Survival is measured relative to the negative control. [e] Minimum mutagenic concentration, minimum concentration at which the sample was statistically significantly mutagenic. − indicates that the sample was not mutagenic at the concentrations tested.

The lower recoveries for acenaphthylene, acenaphthene, and fluorene (the most volatile of the PAH in the mixture) are likely due to evaporative losses during volume reduction.

Mass and Mutagenicity Distribution in Fractions. The percentage of the total extract mass in each fraction is shown in Figure 1. After fractionation on the CN gravity column, 59 ± 0.4% of the total mass was in the A fraction and 34 ± 0.5% was in the B fraction. Some or possibly all of the remaining material appeared as a brown band on the top of the column. Additional losses (4.3% of the total extract mass) occurred in fractionating the A.1 fraction; minor losses occurred during some of the other fractionation steps. Overall, ~86% of the total extract mass was recovered in the 15 fractions.

The mutagenicity testing results, shown in Table 3, indicate that the maximum mutagenicity levels induced by the extract and the fractions were only ~2-fold higher than background, which suggests that the sample did not contain high concentrations of potent mutagens. The response of the positive controls (1 μg/mL B[*a*]P) was on average 4−5-fold higher than background. It was also observed that the MMC values increased disproportionately after the first, third, and fourth fractionation steps. It is not known why this occurred. It is possible that mutagenic compounds could have been lost during fractionation; however, this explanation is inconsistent with the high recoveries we observed for mass and individual mutagens (Table 4). It is also possible that synergistic interactions of sample constituents caused the mutagenicity of the whole extract to be greater than the sum of the mutagenicities of the fractions. This possibility was not investigated. Additional work is needed to understand the apparent loss of mutagenicity observed during fractionation.

The percentage of the mutagenicity in each fraction (normalized to the percentage of the extract mutagenicity attributable to its parent fraction) is shown in Figure 1. After the first fractionation step, ~70% of the total mutagenicity was in the A fraction and ~30% was in the B fraction, indicating that most of the mutagenicity of the whole extract was associated with nonpolar and semipolar compounds rather than with the more polar compounds that eluted in the B fraction. In the second fraction step, the A fraction was separated into three subfractions. The A.1 fraction contained ~20% of the total mutagenicity and 30 ± 0.3% of the total extract mass; the A.2 fraction contained ~50% of the total mutagenicity and 9.2 ± 0.1% of the total extract mass; and the A.3 fraction, which contained 21 ± 4.6% of the extract mass, was not mutagenic even at doses 4-fold higher than the highest equivalent doses at which A.1 and A.2 were tested. Thus, these results indicate that the mutagenicity of the A fraction was entirely associated with nonpolar and semipolar compounds.

At the third fractionation level, the A.1 and A.2 fractions were each separated into two fractions. The A.1.1 fraction contained ~5% of the mutagenicity and 19 ± 1.9% of the mass of the extract, while the A.1.2 fraction contained ~15% of the mutagenicity and 6.7 ± 0.7% of the mass of the extract. The A.2.2 fraction was not mutagenic, and thus it was assumed that the important mutagens in the A.2 fraction were present in the A.2.1 fraction. The A.2.1 and A.2.2 fractions accounted for about 9.1 ± 0.8 and 1.2 ± 0.1% of the extract mass, respectively.

To facilitate chemical analysis, the three mutagenic third-order fractions (A.1.1, A.1.2, and A.2.1) were fractionationed by size-exclusion chromatography. The two fractions produced from each third-order fraction were tested for mutagenicity, and in each case the mutagenicity of the parent third-order fraction was contained in the second or later eluting fourth-order fraction. The A.1.1.2, A.1.2.2, and A.2.1.2 accounted for 0.3 ± 0.1, 2.3 ± 0.2, and 0.8 ± 0.1%, respectively, of the total mass of the extract. Taken together, these results indicate that ~70% of the h1A1v2 cell mutagenicity of the extract was contained in three fourth-order fractions, which represented ~4% of the extract mass.

Chemical Analysis

Fraction A.1.1.2. This fraction was composed almost entirely of PAH and alkyl-substituted PAH. As shown in Table 3, the compounds identified ranged from acenaphthylene (two aromatic rings) to coronene (six aromatic rings). A total of 23 compounds, representing over 80% of the peak area on the GC−MS total ion chromatogram, were quantified. A number of C_1- and C_2-substituted homologues of m/z 178 u (phenanthrene, anthracene), 202 u (e.g., fluoranthene, pyrene), 228 u (e.g., chrysene, benz[*a*]anthracene), and 252

u compounds were also detected, but with the exception of 1-methylphenanthrene and 8-methylfluoranthene, they were not conclusively identified. Based on a comparison of peak areas, the individual C_1- and C_2-substituted homologues were present at levels 5−10-fold lower than the unsubstituted parent PAH. Because of their high mutagenic potency in h1A1v2 cells, 5-methylchrysene and 1-methylbenzo[*a*]pyrene were specifically scanned for but neither was detected (detection limit = ∼10 pg/μL or ∼50 ng/g of particulate material). A number of nitrogen-containing aromatic compounds were detected in this fraction by GC−TEA analysis; however, none of these were conclusively identified.

The most important mutagens in this fraction were cyclopenta[*cd*]pyrene (CPP) and B[*a*]P, accounting for as much as ∼7 and ∼3%, respectively, of the total mutagenicity of the sample (Figure 4). Other compounds in this fraction that contributed to the mutagenicity included benzo[*b*]-fluoranthene (∼0.7%), benzo[*k*]fluoranthene (∼0.1%), benzo-[*ghi*]perylene (∼0.1%), and indeno[1,2,3-*cd*]pyrene (∼0.1%). Benz[*a*]anthracene and chrysene/triphenylene also made minor (<0.1%) contributions to the total mutagenicity. The sum of the individual contributions made by these mutagenic PAH (∼10%) was more than the mutagenicity attributable to the fraction (∼5%), which suggests that inhibition occurred.

Fraction A.1.2.2. This fraction contained many of the same PAH that were identified in the A.1.1.2 fraction as well as some 6-ring $C_{24}H_{14}$ PAH and semipolar PAC. The concentrations of PAH with ≤4 rings were lower in this fraction than in the A.1.1.2 fraction, while the concentrations of PAH with ≥5 rings (with the exception of B[*a*]P) were higher in this fraction than in the A.1.1.2 fraction. As is shown in Table 4, the total concentrations of individual PAH measured in the A.1.1.2 and A.1.2.2 fractions are consistent with what others have reported for this sample.

The $C_{24}H_{14}$ PAH identified included four dibenzopyrene isomers, three naphthopyrene isomers, four naphtho-fluoranthene isomers, benzo[*b*]perylene (B[*b*]Per), and dibenzo[*b*,*k*]fluoranthene (see Table 4). Dibenzo[*a*,*l*]pyrene, the most potent h1A1v2 cell mutagen tested to date, was not detected in the sample. Two of the $C_{24}H_{14}$ PAH (dibenzo-[*e*,*l*]pyrene and naphtho[1,2-*k*]fluoranthene) coeluted, and B[*b*]Per and dibenzo[*a*,*h*]pyrene eluted with other compounds; therefore, only nine individual $C_{24}H_{14}$ PAH were quantified. In addition to $C_{24}H_{14}$ PAH, naphtho[8,1,2-*abc*]-coronene, an 8-ring $C_{30}H_{14}$ compound, was also identified; however, due to its low concentration, it was not quantified. The semipolar PAC identified in this fraction included several ketones (PAK) and quinones (PAQ) and the azaarene benz-[*c*]acridine.

A total of 36 compounds, representing 50% of the peak area of the GC−MS chromatogram of the fraction, were quantified. Of these, 31 compounds have been tested for mutagenicity (see Table 4). As shown in Figure 4, the compounds in this fraction that made the largest contributions to the mutagenicity of the extract were naphtho[2,1-*a*]pyrene (∼3%), benzo[*b*]fluoranthene (∼1%), B[*a*]P (∼1%), dibenzo[*a*,*e*]pyrene (∼1%), and dibenzo[*a*,*i*]pyrene (∼1%). Benzo[*k*]fluoranthene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]-pyrene, dibenz[*a*,*h*]anthracene, naphtho[2,3-*a*]pyrene, and dibenzo[*b*,*k*]fluoranthene each contributed 0.1−1% of the mutagenicity. Approximately two-thirds of the mutagenicity attributable to this fraction (∼10% of the total mutagenicity of the sample) can be accounted for by these 11 PAH. The PAK and PAQ quantified in this fraction did not contribute significantly to the mutagenicity of the sample. 11*H*-Benzo-[*a*]fluoren-11-one and 7*H*-benzo[*c*]fluoren-7-one were not tested for mutagenicity; however, based on the relative inactivity of benzo[*a*]fluorene and benzo[*c*]fluorene in other assays (*18*), it is likely that they would not be significant human cell mutagens. Neither benz[*c*]acridine nor the pesticide methoxychlor [1,1′-(2,2,2-trichloroethylidene)-bis-(4-methoxybenzene)] was mutagenic or significantly toxic to h1A1v2 cells (Table 5). The highest dose at which these compounds were tested (10 μg/mL of cell culture) was over 100-fold greater than their highest concentrations in cell culture resulting from testing the extract and fractions for mutagenicity.

Fraction A.2.1.2. This fraction contained several different classes of oxygenated PAH including PAK, PAQ, anhydrides, coumarins, phenols, aldehydes, and carboxylic acids. A reconstructed GC−MS total ion chromatogram of the fraction is shown in Figure 3. Nine compounds were quantified in the fraction (Table 4). The most abundant was oxy-dichlorophen (5,5′-dichloro-2,2′-dihydroxybenzophenone), an oxidation product of dichlorophen (see Fraction B results). The concentration of oxy-dichlorophen normalized to the mass of unextracted particles was ∼73 ± 7 μg/g. The eight other compounds accounted for ∼40% of the total peak area of the GC−MS chromatogram.

The PAK that were quantified were 1*H*-phenalen-1-one, 4*H*-cyclopenta[*def*]phenanthren-4-one, 11*H*-benzo[*b*]fluoren-11-one, 7H-benz[*de*]anthracen-7-one, and 6*H*-benzo-[*cd*]pyren-6-one. Other PAK that were detected included two isomers of 6*H*-benzo[*cd*]pyren-6-one ($C_{19}H_{10}O$), three isomers of $C_{21}H_{12}O$, and three isomers of $C_{23}H_{12}O$. The PAQ quantified were anthracenequinone and 5,12-naphthacene-quinone; anthanthrenequinone was detected but not quantified. Benzo[*a*]pyrenequinones, which have been identified in air samples (*51*) and are mutagenic to certain strains of bacteria (*52*), were not detected in this fraction.

Three carboxylic acid anhydrides were present in this fraction. 1,8-Naphthalic anhydride was measured at 19 ± 1.5 μg/g, making it the most abundant compound quantified in the airborne particles. Carboxylic acid anhydrides of a 3-ring $C_{14}H_{10}$ PAH (phenanthrene or anthracene) and of a 4-ring $C_{16}H_{12}$ PAH (pyrene or fluoranthene) were also detected, and based on their peak areas, both appeared to be fairly abundant (see Figure 3). One coumarin, 2-nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one, was quantified in the fraction, and several others were detected including a $C_{15}H_8O_2$ coumarin and four $C_{17}H_{10}O_2$ coumarins. A variety of phenols (including seven isomers of $C_{18}H_{12}OH$), PAH aldehydes, and PAH carboxylic acids were also detected, but none of these was conclusively identified.

Eight of the 10 compounds identified in this fraction were tested for mutagenicity, but with the exception of 6*H*-benzo-[*cd*]pyren-6-one, which accounted for ∼0.5% of the mutagenicity of the extract (∼1% of the mutagenicity of the fraction), none of these was significantly mutagenic. Anthanthrenequinone was weakly mutagenic, but due to its relatively low concentration (as suggested by its peak area in Figure 3), it is unlikely to contribute significantly to the mutagenicity of the extract. Oxy-dichlorophen was toxic, inducing 75% killing at the highest dose (10 μg/mL), but it was not mutagenic (Table 5).

Fraction B. This fraction accounted for ∼30% of the mutagenicity of the whole extract. The most abundant compound in the fraction was dichlorophen [2,2′-methylene-bis(4-chlorophenol)], a fungicide that is thought to have been applied to the canvas bags used in the baghouse for sample collection (*32*). Dichlorophen comprised >90% of the peak area of the GC−MS chromatogram. Due to the analytical difficulties presented by this large amount of dichlorophen, the B fraction was not fractionated or further analyzed. Dichlorophen was tested for mutagenicity in h1A1v2 cells but was not mutagenic at concentrations up to 10 μg/mL (Table 4). The concentration of dichlorophen in the particulate material—as measured by HPLC−UV analysis—was 1400 ± 20 μg/g.

TABLE 4. Contribution of PAC and Other Compounds to the Human Lymphoblast Mutagenicity of an Airborne Particle Sample from Washington, DC (SRM 1649)

compound	formula (mw)	ID[a]	Q[b]	concentration (μg/g) A.1.1.2	A.1.2.2	A.2.1.2	total	previous studies[c]	MMC[d]	%Mut[e]
PAH										
acenapthylene	$C_{12}H_8$ (152)	a	d	<0.05	<0.05	ND[f]	<0.1		15000 ± 9500	<0.01
acenaphthene	$C_{12}H_{10}$ (154)	a	d	<0.05	ND	ND	<0.05		NT	
fluorene	$C_{13}H_{10}$ (166)	a	d	0.17 ± 0.01	ND	ND	0.17		NT	
phenanthrene	$C_{14}H_{10}$ (178)	a	d	5.0 ± 0.3	0.13 ± 0.01	ND	5.1	4.5-4.7	NMut (3×10^4)	0
anthracene	$C_{14}H_{10}$ (178)	a	d	0.47 ± 0.03	0.13 ± 0.01	ND	0.60	0.49[k]	NT	
1-methylphenanthrene	$C_{15}H_{12}$ (192)	a	e	0.44 ± 0.03	ND	ND	0.44	0.47[k]	4300 ± 1300	<0.01
fluoranthene	$C_{16}H_{10}$ (202)	a	d	7.9 ± 0.4	<0.05	ND	7.9	6.8−7.3	NMut (10^4)	0
pyrene	$C_{16}H_{10}$ (202)	a	d	6.7 ± 0.4	<0.05	ND	6.7	6.0−7.2	NMut (10^5)	0
benzo[*a*]fluorene	$C_{16}H_{10}$ (216)	a	f	0.53 ± 0.11	ND	ND	0.53		NMut (10^4)	0
benzo[*b*]fluorene	$C_{16}H_{10}$ (216)	a	f	0.12 ± 0.01	ND	ND	0.12	0.32[k]	NMut (10^4)	0
8-methylfluoranthene	$C_{17}H_{12}$ (216)	a	g	0.30 ± 0.02	ND	ND	0.30	0.52[k]	2500 ± 2400	<0.01
benzo[*ghi*]fluoranthene	$C_{18}H_{10}$ (226)	a	h	1.2 ± 0.05	ND	ND	1.2	1.5[l] [g]	NMut (10^4)	0
cyclopenta[*cd*]pyrene	$C_{18}H_{10}$ (226)	a	d	0.37 ± 0.02	ND	ND	0.37	0.41[l]	1.8 ± 1.1	~7
benz[*a*]anthracene	$C_{18}H_{12}$ (228)	a	d	2.6 ± 0.1	0.04 ± 0.001	ND	2.6	2.4−2.8	540 ± 270	~0.07
chrysene[h]	$C_{18}H_{12}$ (228)	a	d	5.1 ± 0.2	0.08 ± 0.01	ND	5.2	3.5−4.2	750 ± 640	~0.05
triphenylene[h]	$C_{18}H_{12}$ (228)	a	h					1.7	2100 ± 1800	~0.07
benzo[*c*]phenanthrene	$C_{18}H_{12}$ (228)	a		ND	ND	ND	ND		240 ± 170	0
5-methylchrysene	$C_{19}H_{14}$ (242)	a		ND	ND	ND	ND		21 ± 13	0
benzo[*e*]pyrene	$C_{20}H_{12}$ (252)	a	h	1.5 ± 0.1	1.8 ± 0.2	ND	3.3	3.3−3.9	8000 ± 640	~0.01
benzo[*b*]fluoranthene	$C_{20}H_{12}$ (252)	a	d	2.1 ± 0.09	3.9 ± 0.4	ND	6.0	6.0−6.2	55 ± 20	~2
benzo[*k*]fluoranthene	$C_{20}H_{12}$ (252)	a	d	0.83 ± 0.05	1.6 ± 0.1	ND	2.4	2.0−2.1	120 ± 71	~0.3
benzo[*j*]fluoranthene	$C_{20}H_{12}$ (252)	a		ND	ND	ND	ND		53 ± 30	0
benzo[*a*]pyrene	$C_{20}H_{12}$ (252)	a	d	1.8 ± 0.08	0.74 ± 0.07	ND	2.5	2.4−3.0	14 ± 9	~4
perylene	$C_{20}H_{12}$ (252)	a	h	0.20 ± 0.01	0.42 ± 0.04	ND	0.62	0.65−0.84	NMut (10^4)	0
1-methylbenzo[*a*]pyrene	$C_{21}H_{14}$ (266)	a		ND	ND	ND	ND		9.4 ± 7.9	0
benzo[*ghi*]perylene	$C_{22}H_{12}$ (276)	a	d	0.69 ± 0.04	2.9 ± 0.4	ND	3.6	3.9−5.2	80 ± 49	~0.6
indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$ (276)	a	d	0.46 ± 0.03	2.5 ± 0.3	ND	3.0	3.3−3.6	50 ± 9.2	~0.8
dibenz[*a,h*]anthracene	$C_{22}H_{14}$ (278)	a	d	ND	0.86 ± 0.06	ND	0.86	0.41−0.45	54 ± 30	~0.2
dibenz[*a,j*]anthracene	$C_{22}H_{14}$ (278)	a		ND	ND	ND	ND	0.27[m]	53 ± 7.1	0
picene	$C_{22}H_{14}$ (278)	a	h	ND	0.30 ± 0.08	ND	0.30	0.39[m]	NMut (3000)	0
benzo[*b*]chrysene	$C_{22}H_{14}$ (278)	a	h	ND	0.38 ± 0.09	ND	0.38	0.26[m]	NMut (3000)	0
coronene	$C_{24}H_{12}$ (300)	a	d	0.16 ± 0.01	1.3 ± 0.4	ND	1.4	3.7[n]	NMut (10^4)	0
dibenzo[*a,l*]pyrene	$C_{24}H_{14}$ (302)	b		ND	ND	ND	ND		0.7 ± 0.6	0
naphtho[2,1-*a*]pyrene	$C_{24}H_{14}$ (302)	b	d	ND	0.88 ± 0.11	ND	0.88		5.0 ± 4.9	~3
naphtho[1,2-*b*]fluoranthene	$C_{24}H_{14}$ (302)	b	j	ND	0.43 ± 0.02	ND	0.43		160 ± 52	<0.05
dibenzo[*b,k*]fluoranthene	$C_{24}H_{14}$ (302)	b	j	ND	0.67 ± 0.05	ND	0.67	0.80[m]	37 ± 10	~0.3
dibenzo[*a,i*]pyrene	$C_{24}H_{14}$ (302)	b	j	ND	0.09 ± 0.01	ND	0.09	0.12[m]	3.8 ± 2.7	~1
dibenzo[*a,e*]pyrene	$C_{24}H_{14}$ (302)	b	j	ND	0.37 ± 0.07	ND	0.37	0.62[m]	5.8 ± 5.7	~1
naphtho[2,3-*a*]pyrene	$C_{24}H_{14}$ (302)	b	j	ND	0.065 ± 0.006	ND	0.065	0.056[m]	1.5 ± 1.1	~0.7
naphtho[2,3-*e*]pyrene	$C_{24}H_{14}$ (302)	b	j	ND	0.30 ± 0.04	ND	0.30	0.24[m]	14 ± 6.7	~0.4
naphtho[2,3-*k*]fluoranthene	$C_{24}H_{14}$ (302)	b	j	ND	0.30 ± 0.07	ND	0.30	0.056[m]	NMut (3000)	0
naphtho[2,3-*b*]fluoranthene	$C_{24}H_{14}$ (302)	b	j	ND	0.12 ± 0.03	ND	0.12	0.22[m]	590 ± 160	<0.01
dibenzo[*e,l*]pyrene[i]	$C_{24}H_{14}$ (302)	b	j	ND	0.37 ± 0.01	ND	0.37		280 ± 180	<0.02
naphtho[1,2-*k*]fluoranthene[i]	$C_{24}H_{14}$ (302)	b	j					0.54[m]	100 ± 71	<0.05
Nitro-PAH										
9-nitroanthracene	$C_{14}H_9NO_2$ (223)	a		ND	ND	ND	ND	0.20[n]	3800 ± 71	0
2-nitrofluoranthene	$C_{16}H_9NO_2$ (247)	a		ND	ND	ND	ND	0.60[o]	280 ± 280	0
3-nitrofluoranthene	$C_{16}H_9NO_2$ (247)	a		ND	ND	ND	ND		5200 ± 2300	0
1-nitropyrene	$C_{16}H_9NO_2$ (247)	a		ND	ND	ND	ND	0.06−0.20[n−q]	540 ± 210	0
2-nitropyrene	$C_{16}H_9NO_2$ (247)	a		ND	ND	ND	ND	0.05[o]	NMut (2×10^4)	0
1,3-dinitropyrene	$C_{16}H_8N_2O_4$ (292)	a		ND	ND	ND	ND		570 ± 40	0
1,6-dinitropyrene	$C_{16}H_8N_2O_4$ (292)	a		ND	ND	ND	ND		58 ± 4	0
1,8-dinitropyrene	$C_{16}H_8N_2O_4$ (292)	a		ND	ND	ND	ND		350 ± 220	0
PAH Ketones										
9-fluorenone	$C_{13}H_8O$ (180)	a	h	ND	1.6 ± 0.11	ND	1.6		NT	
phenalenone	$C_{13}H_8O$ (180)	a	d	ND	ND	1.3 ± 0.08	1.3		5400 ± 800	<0.01
4*H*-Cyclopenta[*def*]phenanthren-4-one	$C_{15}H_8O$ (204)	a	d	ND	0.47 ± 0.06	ND	0.47		NMut (10^4)	0
11*H*-benzo[*a*]fluoren-11-one	$C_{17}H_{10}O$ (230)	a	h	ND	1.9 ± 0.21	ND	1.9		NT	
7*H*-benzo[*c*]fluoren-7-one	$C_{17}H_{10}O$ (230)	a	h	ND	0.71 ± 0.1	ND	0.7		NT	
11*H*-benzo[*b*]fluoren-11-one	$C_{17}H_{10}O$ (230)	a	h	ND	0.91 ± 0.13	4.1 ± 0.34	5.0		NT	
7*H*-benz[*de*]anthracen-7-one	$C_{17}H_{10}O$ (230)	a	d	ND	ND	4.5 ± 0.34	4.5		3300 ± 1800	~0.02

TABLE 4 (Continued)

compound	formula (mw)	ID[a]	Q[b]	concentration (μg/g) A.1.1.2	A.1.2.2	A.2.1.2	total	previous studies[c]	MMC[d]	%Mut[e]
cyclopenta[*cd*]pyren-3(4*H*)-one	$C_{18}H_{10}O$ (242)	a		ND	ND	ND	ND		2600 ± 71	0
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$ (254)	a	d	ND	ND	1.8 ± 0.10	1.8		43 ± 24	~0.5
7*H*-dibenz[*de,j*]anthracen-7-one	$C_{21}H_{12}O$ (280)	a		ND	ND	ND	ND		NMut (10^4)	0
PAH Quinones										
anthracenequinone	$C_{14}H_8O_2$ (208)	a	d	ND	1.3 ± 0.11	1.4 ± 0.12	2.7		NMut (5000)	0
phenanthrenequinone	$C_{14}H_8O_2$ (208)	a		ND	ND	ND	ND		NMut (1000)	0
7,12-benz[*a*]anthracene quinone	$C_{18}H_{10}O_2$ (258)	a	d	ND	2.4 ± 0.25	ND	2.4		NMut (10^4)	0
5,12-naphthacenequinone	$C_{18}H_{10}O_2$ (258)	a	h	ND	ND	1.8 ± 0.11	1.8		NMut (10^4)	0
1,4-chrysenequinone	$C_{18}H_{10}O_2$ (258)	a		ND	ND	ND	ND		NMut (1000)	0
1,6-benzo[*a*]pyrene-quinone	$C_{20}H_{10}O_2$ (282)	a		ND	ND	ND	ND		NMut (300)	0
3,6-benzo[*a*]pyrene-quinone	$C_{20}H_{10}O_2$ (282)	a		ND	ND	ND	ND		NMut (300)	0
4,5-benzo[*a*]pyrene-quinone	$C_{20}H_{10}O_2$ (282)	a		ND	ND	ND	ND		NMut (600)	0
anthanthrenequinone	$C_{22}H_{10}O_2$ (306)	a	d	ND	ND	D	D		920	
Acid Anhydrides										
1,2-naphthalic anhydride	$C_{12}H_6O_3$ (198)	a		ND	ND	ND	ND		NMut (10^4)	0
1,8-naphthalic anhydride	$C_{12}H_6O_3$ (198)	a	h	ND	ND	19 ± 1.5	19		NMut (10^4)	0
anthracene/phenanthrene carboxylic acid anhydride	$C_{16}H_8O_3$ (248)	c		ND	ND	D	D		NT	
pyrene/fluoranthene carboxylic acid anhydride	$C_{18}H_8O_3$ (272)	c		ND	ND	D	D		NT	
Coumarins										
2-nitro-6*H*-dibenzo[*b,d*]-pyran-6-one	$C_{13}H_7O_4N$ (241)	a	d	ND	ND	2.3 ± 0.3	2.3	0.82[q]	NMut (5×10^4)	0
Others										
methoxychlor	$C_{16}H_{15}Cl_3O_2$ (344)	a	d	ND	0.91 ± 0.13	ND	0.91		NMut (10^4)	0
dichlorophen[j]	$C_{13}H_{10}Cl_2O_2$ (268)	b	d	ND	ND	ND	1400 ± 20		NMut (1000)	0
oxy-dichlorophen[k]	$C_{13}H_8Cl_2O_3$ (282)	a	d	ND	ND	73 ± 7.3	73		NMut (10^4)	0
benz[*c*]acridine	$C_{17}H_{11}N$ (229)	a	i	ND	0.26 ± 0.02	ND	0.26	0.21[r]	NMut (10^4)	0
benzo[*b*]naphtho[2,1-*d*]-thiophene	$C_{16}H_{10}S$ (234)	a	f	0.53 ± 0.03	ND	ND	0.53	0.70[k]	NMut (10^4)	0
									total = ~20%	

[a] Identification was made by (a) mathicng the mass spectra and GC retention time with an authentic standard, (b) matching UV−vis spectra and LC retention time with an authentic standard, or (c) matching mass spectra with spectra in mass spectral libraries. [b] Quantification was performed by GC−MS using response factor from (d) an authentic standard, (e) phenanthrene, (f) pyrene, (g) fluoranthene, (h) a standard for a structural isomer, or (i) benz[*a*]anthracene. (j) Quantification was performed by LC−UV using a response factor generated with naphtho[2,1-*a*]pyrene. Concentrations are expressed in units of μg/g of unextracted particles. [c] Concentrations have been reported in ref *35* unless otherwise noted (k, *34*; L, *37*; m, *36*; n, *38*; o, *86*; p, *85*; q, *25*; r, *33*). [d] MMC, minimum mutagenic concentration in ng/mL of human cell culture [MMCs have been published elsewhere (*27*, *28*)]. [e] Percent of the total extract mutagenicity accounted for by compound. [f] ND, compound was not detected. NT, compound was not tested for mutagenicity. NMut, compound was not mutagenic at the dose range tested (highest dose tested in ng/mL cell culture). D, compound was detected, but its concentration was not determined. [g] Benzo[*ghi*]perylene co-eluted with benzo[*c*]phenanthrene. [h] Chrysene and triphenylene co-eluted. %Mut was calculated separately for each compound assuming it comprised 100% of the peak area. [i] Dibenzo[*e,l*]pyrene and naphtho[1,2-*k*]fluoranthene co-eluted. %Mut was calculated separately for each compound assuming it comprised 100% of the peak area. [j] Dichlorophen was measured in the B fraction. Quantification was done by LC−UV using a response factor generated with an authentic dichlorphen standard. [k] 5,5′-Dichloro-2,2′-dihydroxybenzophenone.

Discussion

Human Lymphoblast Mutagens in SRM 1649. Among the compounds that were found to contribute significantly to the h1A1v2 cell mutagenicity of SRM 1649 were PAH with 4−6 rings, including CPP, B[*a*]P, benzo[*b*]fluoranthene (B[*b*]F), naphtho[2,1-*a*]pyrene (N[2,1-*a*]P), dibenzo[*a,e*]-pyrene (DB[*a,e*]P), and dibenzo[*a,i*]pyrene (DB[*a,i*]P). CPP, a potent bacterial mutagen (*53*, *54*) and rodent carcinogen (*55*, *56*), was nearly seven times more mutagenic than B[*a*]P in h1A1v2 cells, making it one of the most mutagenic PAH tested in this cell line (*27*). Therefore, despite its relatively low concentration in the sample (~0.4 ppm), it is not surprising that CPP was found to be an important mutagen. CPP has also been identified as an important mutagen in combustion emissions. Skopek et al. (*57*) reported that CPP accounted for ~8% of the +S9 mutagenicity in human (HH-4) cells induced by kerosene soot extracts. Because CPP is widely distributed in urban air (*58*−*60*), it is likely to be an important human cell mutagen in airborne particles from other urban areas. In a recent study, Hannigan et al. (*61*) reported that CPP could account for as much as ~4% of the human cell mutagenicity of airborne particles from Los Angeles. B[*a*]P and B[*b*]F are also ubiquitous mutagens in urban air (*18*, *22*, *62*). B[*a*]P has been reported to account for 2−4% of the +S9 *S. typhimurium* mutagenicity in airborne particle samples (*63*). B[*b*]F, which is typically present in airborne particles at concentrations that are comparable to those of B[*a*]P (*60*, *64*), is generally less mutagenic than B[*a*]P (*65*, *66*); therefore, B[*b*]F would be expected to account for a smaller fraction of the total mutagenicity of airborne particles than B[*a*]P.

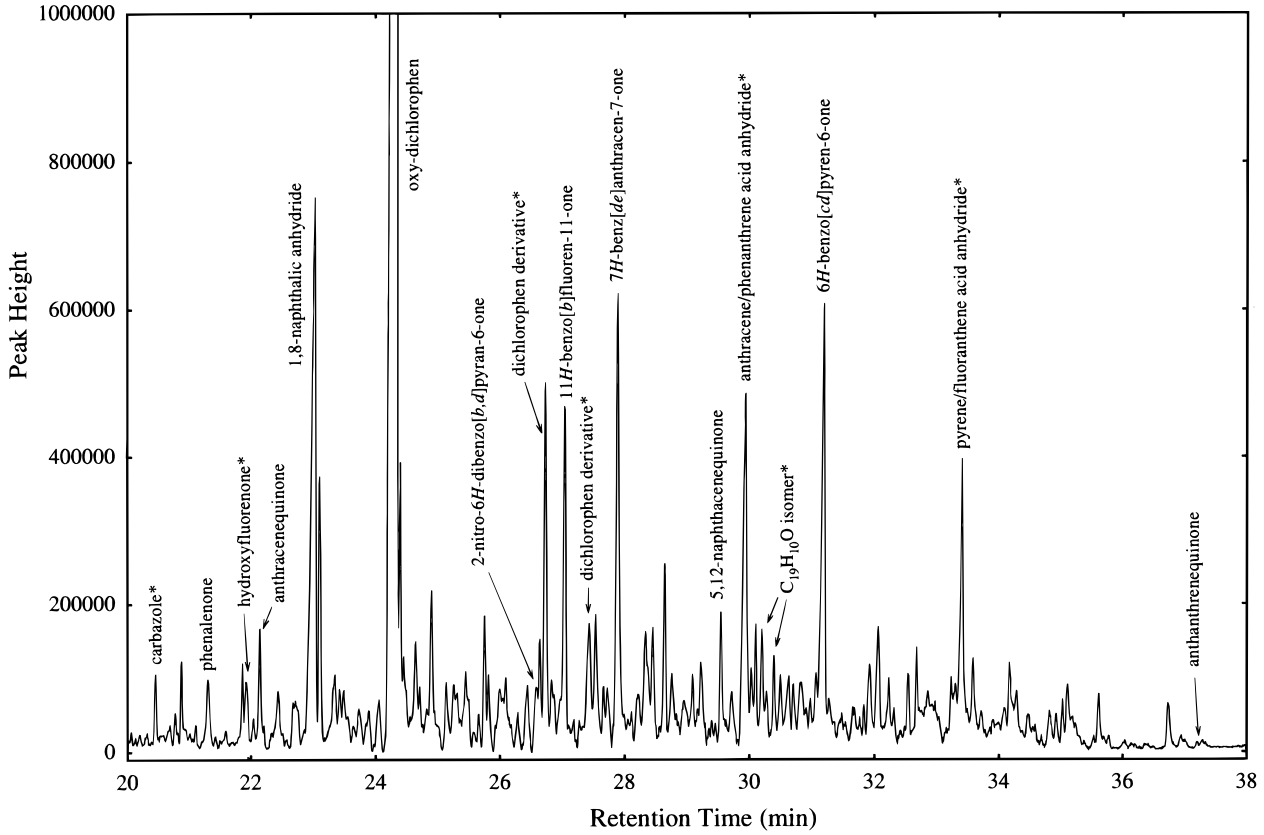

FIGURE 3. Reconstructed GC−MS total ion chromatogram for fraction A.2.1.2. Compounds were identified by matching GC retention times and mass spectra to those of authentic standards except for those marked with an asterisk, which were only tentatively identified. Oxy-dichlorophen (5,5′-dichloro-2,2′-dihydroxybenzophenone) is an oxidation product of the fungicide dichlorophen, which is thought to have been used to treat the canvas bags in the baghouse samplers.

To date, relatively little work has been done to determine the concentrations of 6-ring $C_{24}H_{14}$ PAH in urban airborne particles. Part of the reason for this is that there are many isomers [e.g., Schmidt et al. (*67*) proposed structures for 33 $C_{24}H_{14}$ homologues of fluoranthene, pyrene, and perylene and 22 $C_{24}H_{14}$ homologues of aceanthrylene and acephenanthrylene], but relatively few have been synthesized and even fewer are commercially available. In addition, some $C_{24}H_{14}$ PAH coelute during gas and liquid chromatographic separation, most have identical mass spectra, and their concentrations in airborne particles are typically low (≤1 ppm). Despite these limitations, some measurements have been reported. Wise et al. (*68*) reported that the concentrations of DB[*a,e*]P, DB[*a,l*]P, and other unidentified dibenzopyrene and dibenzofluoranthene isomers ranged from 1.5 to 5.7 μg/g in an air sample from Philadelphia. In analyzing samples from New Jersey, Greenberg et al. (*69*) found that the concentrations of DB[*a,e*]P and DB[*a,l*]P were comparable to those of B[*a*]P, while the concentrations of dibenzo[*b,k*]-fluoranthene (DB[*b,k*]F) were approximately 4-fold lower than those of B[*a*]P. Recently, Wise et al. (*36*) quantified nine $C_{24}H_{14}$ PAH in SRM 1649. Our results here are generally in agreement with what Wise et al. report (see Table 4); however, we also found benzo[*b*]perylene, dibenzo[*e,l*]pyrene, naphtho[1,2-*b*]fluoranthene (N[1,2-*b*]F), and N[2,1-*a*]P. To our knowledge, this is first time that N[1,2-*b*]F has been identified in an airborne particle sample. Also, to our knowledge, this is the first study to report that N[2,1-*a*]P, which accounted for ∼3% of the mutagenicity of the extract, is a significant environmental mutagen. In addition to N[2,1-*a*]P, we also identified N[2,3-*a*]P in the sample. The minimum mutagenic concentration of N[2,3-*a*]P is roughly 7-fold lower than that of B[*a*]P, making it one of the most potent h1A1v2 cell mutagens tested to date (*28*); however, due to its relatively low concentration in the sample (∼65 ng/g), N[2,3-*a*]P could account for only ∼0.7% of the total mutagenicity. As a group, $C_{24}H_{14}$ PAH accounted for ∼33% of the mutagenicity of the PAH fractions or ∼6% of the mutagenicity of the whole extract.

In addition to 4−6-ring PAH, we also found that compounds eluting in the semipolar (A.2) and polar (B) fractions of the extract accounted for significant amounts of the total mutagenicity (∼50% and ∼30%, respectively). While no potent mutagens were identified, we cannot rule out the possibility that the mutagenicity of these fractions is accounted for by a small number of very potent mutagens, nor can we rule out the possibility that this mutagenicity is accounted for by many weak mutagens. In the semipolar fraction, the PAK 6*H*-benzo[*cd*]pyren-6-one (BPK) was the most important mutagen identified, accounting for as much as ∼0.5% of the total mutagenicity of the extract. Two isomers of BPK and isomers of other higher molecular weight compounds that had mass spectra characteristic of PAK were also detected in the A.2 fraction, but due to the lack of analytical standards, we were unable to conclusively identify any of these. On the basis of our finding with BPK, it is possible that these (or other) higher molecular weight (i.e., ≥ mw 254 u) PAK could be important mutagens in the sample. Dicarboxylic acid anhydrides of three and four aromatic ring PAH were also tentatively identified in the A.2 fraction. In finding that pyrene-3,4-dicarboxylic acid anhydride accounted for a small (but unquantified) amount of the total −S9 mutagenicity (TA98) in a diesel exhaust, Rappaport et al. (*70*) speculated that other unidentified PAH dicarboxylic acid anhydrides were among the important mutagens in the sample. While it is possible that the dicarboxylic acid anhydrides of 3- and 4-ring PAH in the A.2 fraction could be important mutagens, the likelihood seems remote based on what we have observed previously. In testing a series of

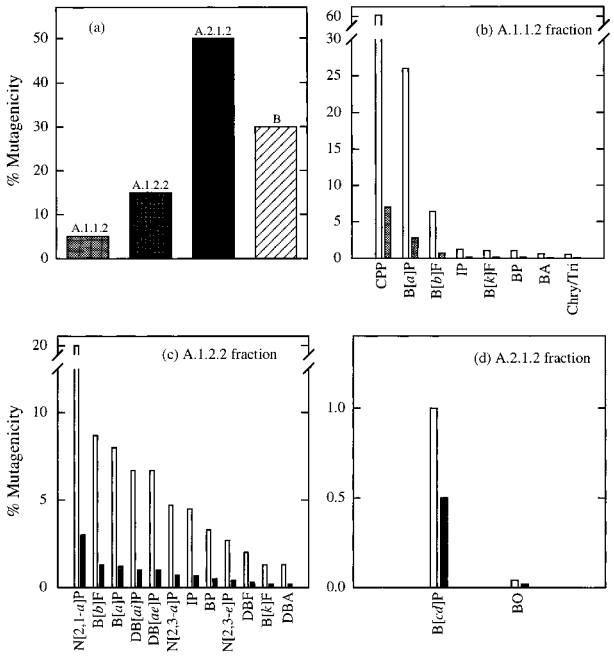

FIGURE 4. Percentage of human lymphoblast mutagenicity attributable to different fractions (a) and to individual compounds within those fractions. In panels b−d the open and shaded boxes represent the percentage of the fraction mutagenicity and total extract mutagenicity, respectively, attributable to each compound: cyclopenta[*cd*]pyrene, (CPP); benz[*a*]anthracene, (BA); chrysene/triphenylene, (Chry/Tri); benzo[*e*]pyrene, (B[*e*]P); benzo[*b*]fluoranthene, (B[*b*]F); benzo[*k*]fluoranthene, (B[*k*]F); benzo[*a*]pyrene, (B[*a*]P); benzo[*ghi*]perylene, (BP); indeno[1,2,3-*cd*]pyrene, (IP); dibenz[*a,h*]-anthracene, (DBA); dibenzo[*a,e*]pyrene, (D[*a,e*]P); dibenzo[*a,i*]pyrene, (D[*a,i*]P); naphtho[2,1-*a*]pyrene, (N[2,1-*a*]P); naphtho[2,3-*a*]pyrene, (N[2,3-*a*]P); naphtho[2,3-*e*]pyrene, (N[2,3-*e*]P); dibenzo[*b,k*]fluoranthene, (DBF); 7*H*-benz[*de*]anthracene-7-one, (BO); 6*H*-benzo[*cd*]pyren-6-one, (B[*cd*]P).

PAK, polycyclic aromatic quinones, coumarins, and dicarboxylic acid anhydrides for mutagenicity in h1A1v2 cells, only the PAK as a group were significantly mutagenic (*27*).

Comparison to Other Studies. Many investigators have used bioassay-directed chemical analysis techniques to identify *S. typhimurium* mutagens in airborne particles. For example, Siak et al. (*71*) tested samples from the Detriot area and found that approximately half of the −S9 mutagenicity in strain TA98 was attributable to fractions containing PAH, mononitro-PAH, and dinitro-PAH. The remainder of the activity was present in more polar fractions. The mutagenic activity in all of the fractions was found to decrease significantly when tested in nitroreductase deficient strains of TA98, suggesting that the most potent mutagens were nitro-substituted PAH. Only 3% of the mutagenicity was attributable to 1-nitropyrene and 1,6- and 1,8-dinitropyrene. de Raat et al. (*72*) reported that ambient aerosols from urban sites in The Netherlands contained seven distinct groups of bacterial mutagens. One group contained PAH and could account for as much as 5−20% of the +S9 mutagenicity depending on the strain of *S. typhimurium* being used. A second group that contained mononitro-PAH accounted for 12−14% of the +S9 mutagenicity and 13−24% of the −S9 mutagenicity. The five other groups were characterized by more polar compounds. Dinitro-PAH were not detected in these samples and were thus discounted as being important mutagens. In a previous study with SRM 1649, Lewtas et al. (*29*) reported that PAH contributed at most 8% of the +S9 mutagenicity in TA98, while nitro-PAH contributed only ~6% of the −S9 mutagenicity. Of the remaining mutagenicity, semipolar ("polar neutral") fractions contributed 30−40%, and the organic acid fraction contributed 50% of the −S9 and 65% of the +S9 mutagenicity. This general trend of finding the majority of the bacterial mutagenicity in more polar fractions of airborne particle extracts has also been reported by others (*73*−*77*) and is consistent with what we observed in testing fractions of SRM 1649 for mutagenicity in human h1A1v2 cells.

TABLE 5. Human Lymphoblast Mutagenicity of Previously Untested Compounds Identified in SRM 1649

compound[a]	dose[b] (ng/mL)	mutant fraction ± SD[c] (× 10^6)	relative survival[d]	result[e]
methoxychlor	0	23 ± 4	1.0	
AC (95%)	1	24 ± 6	0.99	
	10	26 ± 0.8	1.2	−
	1000	33 ± 2	0.89	
	10000	22 ± 8	0.80	
	PC	200 ± 30	0.38	
benz[*c*]acridine	0	16 ± 6	1.0	
PR (99.5%)	10	14 ± 0	0.99	
	100	13 ± 0.1	1.0	−
	10000	22 ± 4	0.46	
	PC	160 ± 11	0.28	
dichlorophen	0	22 ± 7	1.0	
AS (>99.5%)	1	16 ± 9	1.1	
	10	19 ± 3	1.0	
	1000	19 ± 5	0.79	−
	10000	NP[g]		
	PC	160 ± 51	0.43	
oxy-dichlorophen[f]	0	25 ± 7	1.0	
OR (>99.5%)	10	16 ± 4	0.96	−
	100	20 ± 5	1.2	
	1000	27 ± 13	1.0	
	10000	34 ± 9	0.25	
	PC	270 ± 69	0.39	

[a] AC, Aldrich Chemical Co., Milwaukee, WI; AS, AccuStandard Inc., New Haven, CT; OR, Oryza Laboratories, Inc., Newburyport, MA; PR, PAH Research Institute, Greifenberg, Germany. Compound purities, as reported by the manufacturers, are shown in parentheses, except for benz[*c*]acridine, which was determined as part of this study. [b] Nanograms of compound per milliliter of cell culture; PC, positive control (1 μg of B[*a*]P/mL of cell culture). Historical PC = 210 ± 80 (n = 130). [c] Results represent the mean of two independent experiments (±SD) with the exception of oxy-dichlorophen for which n = 4. [d] Survival is measured relative to the negative controls. [e] − indicates that the compound was not mutagenic at the doses tested. [f] 5,5′-Dichloro-2,2′-dihydroxybenzophenone. [g] NP, cultures were not plated due to excessive toxicity.

Possible Role of Nitro-PAH. Although studies with *S. typhimurium* indicate that polar fractions account for a significant portion of the total mutagenicity of airborne particle extracts, relatively few potent polar mutagens have been identified. Some investigators have attributed the mutagenicity in these fractions to unidentified NO_2-substituted PAH (*76*, *78*). Others have implicated nitro-PAH lactones, hydroxylated nitro-PAH (HN-PAH) and azaarenes. Helmig et al. (*25*) reported that the nitro-PAH lactone, 2-nitro-6*H*-dibenzo[*b,d*]pyran-6-one (2-NDBP), could account for nearly 50% of the total −S9 mutagenicity (TA98) of aerosol samples from Claremont, CA. Nishioka et al. (*24*) suggested that HN-PAH, in particular hydroxylated nitropyrenes and nitrofluoranthenes, were among the most important bacterial mutagens present in samples from Philadelphia. Matsumoto and Inoue (*74*) found that the semipolar fraction of an Osaka sample, which was highly mutagenic to bacteria, contained azaarenes, low levels of PAH, and many different classes of oxy-PAH including ketones, quinones, coumarins, and aldehydes. Although no known potent mutagens were present at levels high enough to account for a significant portion of the observed mutagenicity, it was concluded that azaarenes were the most important mutagens in the fraction despite their relatively low concentrations.

One potential limitation of using *S. typhimurium* to identify mutagens in airborne particles is that the bacterium is particularly sensitive to nitro-aromatic compounds. Rosenkranz and Mermelstein (*79*) observed that 1-nitropyrene (1-NP), 1,3-dinitropyrene (1,3-DNP), 1,6-dinitropyrene (1,6-DNP), and 1,8-dinitropyrene (1,8-DNP) were 200, 6.3E4, 8.0E4, and 1.1E5 times, respectively, more mutagenic (mutants/unit mass) in *S. typhimurium* strain TA98 than the potent mutagen benzo[*a*]pyrene (B[*a*]P). In this experiment, the nitro-PAH were tested in the absence of rat liver enzymes (S9), while B[*a*]P was tested in the presence of S9. It has been demonstrated that *S. typhimurium* contain endogenous nitro-reductase enzymes that are extremely efficient in converting nitro-aromatic compounds into aryl-hydroxylamines, the ultimate mutagenic metabolites of nitro-PAH (*80*). When 1-NP and the three dinitropyrene isomers were tested in *S. typhimurium* strains deficient in nitro-reductase and dinitro-reductase enzymes, respectively, their mutagenicity was substantially reduced (*81*). Likewise, when these same nitro-PAH were tested in *S. typhimurium* in the presence of S9, a 10-fold reduction in the mutagenicity of 1-NP and about a 1000-fold reduction in the mutagenicity of the dinitropyrenes was observed (*82*−*84*).

In contrast to what has been observed in testing extracts of airborne particles for mutagenicity in bacteria, we did not find nitrogen-containing compounds to be important human lymphoblast mutagens in SRM 1649. 2-NDBP, the potent −S9 TA98 mutagen, was present at a relatively high concentration in the sample (2.3 ± 0.3 μg/g), but because it was only weakly mutagenic in h1A1v2 cells, it accounted for <0.01% of the total mutagenicity of the sample. Previously, it was reported that 9-nitroathracene (9-NA), 2-nitrofluoranthene (2-NF), 1-NP, 2-nitropyrene (2-NP), 1,3-DNP, 1,6-DNP, and 1,8-DNP were less mutagenic in this cell line than B[*a*]P (*27*). For these compounds to be important h1A1v2 cell mutagens, their concentrations would need to be on the order of ∼1 μg/g or higher; however, none of these nitro-PAH or dinitropyrenes were detected in the sample (limit of detection ∼100 ng/g). It should be noted that 9-NA, 2-NF, 1-NP, and 2-NP concentrations in the sub-microgram per gram range have been reported for SRM 1649 (see Table 4), which suggests that either they were lost during fractionation or our analytical method was insufficiently sensitive to detect them. Benz[*c*]acridine, the only other nitrogen-containing compound quantified in the sample, was not mutagenic in h1A1v2 cells over the dose range of 0.01−10 μg/mL, and thus, it did not contribute to the mutagenicity of the sample. Additional work is required to determine whether dibenzacridines, which are known to be carcinogenic in animal assays (*18*) and have been reported in urban air (*87*), are important h1A1v2 cell mutagens in SRM 1649. Likewise, additional research is also needed to determine whether HN-PAH contribute significantly to the mutagenicity of this sample.

Differences in complements of metabolizing enzymes could explain why nitrogen-containing PAC are potent mutagens to *S. typhimurium* strains but not to h1A1v2 cells. As noted above, *S. typhimurium* contain endogenous reductase enzymes that are very efficient in converting nitro-aromatic compounds into mutagenic metabolites (*80*). h1A1v2 cells, by contrast, constitutively express cytochrome P4501A1 (CYP1A1) at levels ∼50-fold higher than the basal level and 3-fold higher than the fully induced level of the parent cell line. While we have shown that nitro-PAH are mutagenic to h1A1v2 cells, the metabolism of nitro-PAH by CYP1A1 is not well understood. For example, we do not know the extent to which altering the levels of CYP1A1 in these cells would affect the mutagenicity of nitro-PAH. Thus, it is possible that, in human cell lines expressing lower levels of CYP1A1 and/or higher levels of other enzymes, nitro-PAH could be more mutagenic than in h1A1v2 cells.

Relevance to Human Health. While our results shed light on the kinds and amounts of human lymphoblast mutagens present in urban airborne particles, caution should be used in extrapolating these findings to humans. The are several reasons for this. One is that the sample studied, SRM 1649, does not accurately represent the airborne particles to which urbanites are typically exposed: (i) as noted previously, the sample contains large amounts of the fungicide dichlorophen and some derivative compounds [although we found that dichlorophen and a major derivative, oxy-dichlorophen, were not mutagenic or toxic to h1A1v2 cells, even at very high doses, we cannot rule out the possibility that other dichlorophen derivatives (or possibly other artifact chemicals) may have contributed to the observed genotoxicity of the sample]; (ii) SRM 1649 was collected nearly 20 years ago, and thus, it is possible that chemical constituents may have degraded during this long period in storage; and (iii) because the sample was collected using a sampler that did not segregate the particles based on size, SRM 1649 contains both respirable particles (i.e., those particles < 2−3 μm in diameter) and larger, nonrespirable particles. Interestingly, despite these drawbacks of using SRM 1649, our results are consistent with those of Hannigan et al. (*61*), who used h1A1v2 cells and a bioassay-directed chemical analysis method similar to ours to analyze a sample of respirable particles collected in Los Angeles in 1993. Hannigan et al. reported that as much as 17% of the mutagenicity of this sample was attributable to fractions enriched in PAH. The compounds that made the largest contributions to the mutagenicity of the PAH fractions included CPP, B[*a*]P, B[*b*]F, B[*k*]F, benzo[*ghi*]perylene, and indeno[1,2,3-*cd*]pyrene. Potent h1A1v2 cell mutagens identified in semipolar fractions of the Los Angeles sample included 2-NF and 6*H*-benzo[*cd*]pyren-6-one.

Another reason that caution should be used in extrapolating our findings to humans is that we do not know whether h1A1v2 accurately reflect the behavior of normal lymphoblastoid cells in vivo. For example, when human cells are cultured, they typically lose their ability to express cytochrome P450s. To compensate for this, h1A1v2 cells have been genetically engineered to constitutively overexpress CYP1A1. However, it is not known whether the levels of CYP1A1 in h1A1v2 cells are comparable to levels expressed in normal human cells. It is also not known whether the h1A1v2 cell line is deficient in other enzyme systems (e.g., DNA repair enzymes) that could be important in chemical mutagenesis. Thus, due to these uncertainties concerning the h1A1v2 cell line, the compounds we have identified as important mutagens may not necessarily pose the same risks to lymphoblastoid or other human cells in vivo. Nonetheless, these findings expand our knowledge of the kinds of chemicals in airborne particles that can mutate human cells, and they form the basis for ongoing studies to identify other important human lymphoblast mutagens in samples of respirable particles.

Acknowledgments

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Author-Supplied Registry Numbers: Acenaphthylene (208-96-8); phenanthrene (85-01-8); 1-methylphenanthrene (832-69-9); fluoranthene (206-44-0); pyrene (129-00-0); benzo[*a*]fluorene (238-84-6); benzo[*b*]fluorene (243-17-4); 8-

methylfluoranthene (20485-57-8); cyclopenta[*cd*]pyrene (27208-37-3); benzo[*ghi*]fluoranthene (203-12-3); benz[*a*]-anthracene (56-55-3); chrysene (218-01-9); triphenylene (217-59-4); benzo[*b*]naphtho[2,1-*d*]thiophene (239-35-0); benzo-[*e*]pyrene (192-97-2); benzo[*b*]fluoranthene (205-99-2); benzo[*k*]fluoranthene (207-08-9); perylene (198-55-0); benzo-[*a*]pyrene (50-32-8); benzo[*ghi*]perylene (191-24-2); indeno-[1,2,3-*cd*]pyrene (193-39-5); dibenz[*a*,*h*]anthracene (53-70-3); dibenz[*a*,*j*]anthracene (224-41-9); picene (213-46-7); benzo[*b*]chrysene (214-17-5); coronene (191-07-1); dibenzo-[*a*,*e*]pyrene (192-65-4); dibenzo[*a*,*i*]pyrene (189-55-9); diben-zo[*e*,*l*]pyrene (192-51-8); naphtho[2,1-*a*]pyrene (189-96-8); naphtho[2,3-*a*]pyrene (196-42-9); naphtho[2,3-*e*]pyrene (193-09-9); dibenzo[*b*,*k*]fluoranthene (205-97-0); naphtho(2,3-*b*)-fluoranthene (206-06-4); naphtho[1,2-*k*]fluoranthene (238-04-0); naphtho[2,3-*k*]fluoranthene (207-18-1); naphtho[2,3-*j*]fluoranthene (205-83-4); 9-fluorenone (486-25-9); phenalenone (548-39-0); 4*H*-cyclopenta[*def*]phenanthrene-4-one (5737-13-3); anthraquinone (84-65-1); 1,8-naphthalic anhydride (81-84-5); 7*H*-benz[*de*]anthracen-7-one (82-05-3); 2-nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one (6623-66-1); 6*H*-benzo[*cd*]pyren-6-one (3074-00-8); 7,12-benz[*a*]anthracene-quinone (2498-66-0); 5,12-naphthacenequinone (1090-13-7); anthanthrenequinone (641-13-4); 2-nitrofluorene (607-57-8); dichlorophen (85-01-8); 5,5′-dichloro-2,2′-di-hydroxybenzophenone (6178-89-8); methoxychlor (72-43-5); benz[*c*]acridine (225-51-4).

Supporting Information Available

One table giving complete mutagenicity testing results for the whole extract and all 15 fractions (6 pages) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the Supporting Information from this paper or microfiche (105 × 148 mm, 24× reduction, negatives) may be obtained from Microforms Office, American Chemical Society, 1155 16th St. NW, Washington, DC 20036. Full bibliographic citation (journal, title of article, names of authors, inclusive pagination, volume number, and issue number) and prepayment, check or money order for $16.50 for photocopy ($18.50 foreign) or $12.00 for microfiche ($13.00 foreign), are required. Canadian residents should add 7% GST. Supporting Information is available to subscribers electronically via the World Wide Web at URL http://www.chemcenter.org. Users should select Electronic Publications and then Environmental Science and Technology under Electronic Editions. Detailed instructions for using this service, along with a description of the file formats, are available at this site. To download the Supporting Information, enter the journal subscription number from your mailing label. For additional information on electronic access, send electronic mail to si-help@acs.org or phone (202)872-6333.

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