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Bioassay-Directed Chemical Analysis of Los Angeles Airborne Particulate Matter Using a Human Cell Mutagenicity Assay

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The human cell mutagenicity of Los Angeles airborne fine particulate matter is examined via bioassay-directed chemical analysis. A 1993 composite fine particle sample is separated via liquid chromatography into fractions containing organic compounds of varying polarity. Samples are analyzed by the h1A1v2 human cell mutagenicity assay to identify those fractions that contain human cell mutagens and by GC/MS to identify the chemical character of those mutagens. Those subfractions that contain unsubstituted polycyclic aromatic compounds (PAC) are responsible for a considerable portion of the mutagenic potency of the whole atmospheric sample. Six unsubstituted PAC (cyclopenta[*cd*]pyrene, benzo[*a*]pyrene, benzo[*ghi*]-perylene, benzo[*b*]fluoranthene, indeno[1,2,3-*cd*]pyrene, and benzo[*k*]fluoranthene) account for most of the mutagenic potency that can be assigned to specific compounds within the atmospheric samples. Important semipolar mutagens that are quantified include 2-nitrofluoranthene and 6*H*-benzo[*cd*]pyren-6-one. A large number of other aromatic organics are identified as candidates for future testing as pure compounds in the human cell assay, at which time it should be possible to account for more of the mutagenic potency of the atmospheric samples.

Introduction

Organic particulate matter filtered from ambient air has repeatedly been shown to be mutagenic to bacteria (*1*−*4*), mutagenic to human cells (*5*), and carcinogenic (*6*, *7*). Hemminki et al. (*8*) report an increase in the smoking-adjusted risk of lung cancer among urbanites of up to 1.5 times that of rural residents, which adds to the concern that urban air may contain significant concentrations of mutagens and carcinogens.

Particulate air pollution is a complex mixture of thousands of chemical species. It is virtually impossible to identify every chemical species and then determine if that compound is a mutagen. However, much can be done to gauge the general character of the important mutagens in an atmospheric particulate matter sample. In an attempt to isolate the important chemical mutagens in an environmental sample, previous researchers (*9*−*20*) have developed and refined a technique aptly named bioassay-directed chemical analysis. Bioassay-directed chemical analysis involves separating a sample into coherent pieces, called fractions, that contain organic chemicals of similar functionality and polarity. The fractions are tested in a bioassay to determine their mutagenic potency, and the bioassay results are used to direct attention to detailed chemical analysis of those fractions in which the most important mutagens have been isolated. This process of separating and testing can be repeated until the chemical complexity of each mutagenic fraction has been reduced to the point where a fairly comprehensive chemical analysis of the mutagenic fractions can be completed.

Researchers have attempted a myriad of variations on the theme of chemical separation within the context of bioassay-directed chemical analysis, but the primary bioassay used in the past always has been a bacterial mutation assay. When studying diesel engine exhaust, Nishioka et al. (*21*) found that nitro polycyclic aromatic compounds (nitroPAC) accounted for 20−25% of the bacterial mutagenic activity observed without further enzymatic activation of the assay (i.e., absent the addition of post-mitochondrial supernatant preparation, a test condition called −PMS or −S9). Salmeen et al. (*22*) found that mono- and dinitro polycyclic aromatic hydrocarbons (mono- and dinitroPAC) account for 30−40% of the bacterial mutagenicity (−S9) of diesel engine exhaust particles. The hunt for important mutagens then moved from emission sources to ambient air pollution as Wise et al. (*4*) found significant bacterial mutagenic activity (−S9) due to nitroPAC in ambient air, Arey et al. (*23*) found that nitroPAC accounted for 1−8% of the bacterial mutagenic activity (−S9), and Helmig et al. (*24*) concluded that a specific nitroPAC (2-nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one) accounted for ∼45% of the bacterial mutagenic activity of their ambient samples (−S9). Abundant literature exists detailing the significance of nitroPAC as bacterial mutagens.

Bioassay-directed chemical analysis for mutagens has seldom been attempted using biological end points other than bacterial assays, although a few studies do exist. Grimmer et al. (*10*, *25*, *26*) studied extracts of the particulate matter from several air pollutant emission sources using carcinogenic effects in rats as an end point and found unsubstituted PAC with more than three rings to account for the total carcinogenic activity. Using a human cell forward mutation assay in which rat liver provided the catalyst for xenometabolism (the TK-6 cell line), Skopek et al. (*27*) found that 8% of the observed activity of kerosene soot was due to cyclopenta[*cd*]pyrene (CPP). Using the same assay, Barf-knecht et al. (*28*) report that a significant fraction of the activity of diesel engine exhaust is due to fluoranthene. Durant et al. (*20*) found that benzo[*a*]pyrene (BaP) accounted for as much as 50% of the activity of the organics extracted from urban pond sediment in the MCL-5 human cell line, which expresses human xenometabolizing enzymes.

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When comparing the results of studies using bacterial mutation assays to the few studies conducted on data of various air pollution source effluents using either cultured human cells or whole mammalian animals (e.g., mice), a pattern seems to emerge that deserves further investigation. The bacterial assays appear to be reacting to the nitroPAC content of the samples while the mammalian cells including human cells seem to be most affected by the unsubstituted PAC content of the samples. Even though nitroPAC are mutagenic in the human cell assays (*29*), their concentration-weighted activity in typical complex mixtures may be lower than that of the concentration-weighted activity of the unsubstituted PAC in a human cell assay because nitroPAC are present at trace levels by comparison to the unsubstituted PAC. The outcome of the bioassay-directed chemical analysis of complex mixtures seems to be extremely dependent on the biological end point used. The above hints that the most important mutagens affecting human cells may be different than previously inferred from bacterial assays are obtained from a few studies of air pollutant source materials and not from an examination of actual ambient air samples. The ambient air is more complex than the source material because it also contains nitroPAC and oxyPAC that are formed by atmospheric reactions (*30*). To date, the character of the most important human cell mutagens in the urban atmosphere remains to be determined, as no bioassay-directed chemical analyses have been published to date based on samples taken at community routine air monitoring stations.

The present paper seeks to characterize the most important mutagens present in urban airborne particulate matter using bioassay-directed chemical analysis that is based on a human cell assay for gene mutation. A fine particulate matter sample representative of long-term exposure conditions in southern California is created by compositing a portion of every filter sample collected during a 1993 air monitoring campaign, which consisted of 24-h sampling every sixth day for the entire year at four urban locations in southern California. The human cell mutation assay used in this study (*31*) tests mutagenic activity at the thymidine kinase locus in h1A1v2 cells using a 72-h exposure. The h1A1v2 cells are AHH-1 TK+/− cells bearing the plasmid pHSRAA. The plasmid pHSRAA contains two copies of the human CYP1A1 cDNA and confers resistance to 1-histidinol. This cell line has been shown to be sensitive to both PAC and nitroPAC (*32*) and has been used previously to investigate the seasonal and spatial variation of the human cell mutagenicity of fine organic aerosol in southern California (*5*). To the best of our knowledge, this study reports the first bioassay-directed chemical analysis of airborne particulate matter samples taken at community air monitoring stations using a human cell assay.

Experimental Section

Sample Collection. An airborne fine particulate matter sample is created by compositing a portion of every urban fine particulate filter sample collected during a 1993 southern California air monitoring campaign. This air monitoring campaign is described briefly below, and a more detailed description can be found elsewhere (*5, 33*). The air sampling network consists of four urban sites that were selected because each has a different characteristic exposure to ambient aerosol sources, plus a background site located on an offshore island upwind of Los Angeles. Samples taken at the background site are not included in the urban aerosol composite examined in the present paper. The four urban sites included central Los Angeles, which is surrounded by freeways and experiences high concentrations of motor vehicle emissions; Long Beach, which is located in close proximity to the direct emissions from industrial sources such as power plants, petroleum refineries, and the Los Angeles−Long Beach harbor complex; Azusa, which is generally downwind of central Los Angeles and is characterized by relatively high levels of ozone and secondary aerosol that is formed by atmospheric chemical reactions; and Rubidoux, which is located farther downwind and generally receives even more secondary aerosol than Azusa. All air monitoring equipment were located at the South Coast Air Quality Management District (SCAQMD) air monitoring stations in the communities named. The ambient particulate matter samples were taken for 24 h every sixth day for the entire year of 1993 at these four urban air monitoring stations. Quartz fiber filters (102 mm diameter Pallflex Tissuquartz 2500 QAO) were used for the particulate matter collection. All quartz fiber filters were prebaked for at least 6 h prior to use at 750 °C to lower their carbon blank. Each filter was loaded the day prior to sampling and unloaded on the day after sampling. Field and laboratory blanks were also taken to ensure that there was minimal contamination of the ambient sampling system. The filters were transported to the sampling sites in prebaked aluminum foil and returned to the laboratory in annealed glass jars with solvent-washed Teflon-lined lids. All filters were stored in a freezer at −21 °C.

A high-volume dichotomous virtual impactor, described in detail by Solomon et al. (*34*), was used at each site to collect the fine particulate matter (particle diameter $d_p < 3$ μm) samples used for this study. This sampler has the ability to gather a large quantity of size-separated organic aerosol in a 24-h period. One-sixth of each fine particle filter collected was used to make a 1993 urban composite that physically represents the annual average aerosol concentration and composition averaged over all four urban sites. Filter allocation is described in detail elsewhere (*5*). The human cell mutagenicity assay used here requires approximately 500 μg of organic carbon per test, and generally, duplicate tests are performed. All sample organic aerosol mass is reported in units of micrograms of equivalent organic carbon (EOC), which is defined as the amount of organic carbon present in the filter composite prior to extraction as determined by thermal evolution and combustion analysis of separate sections cut from the same quartz fiber filters (*5*). The 1993 urban composite assembled for bioassay-directed chemical analysis contained 84 mg of EOC, thus providing enough organic material to permit multiple level separation procedures designed to isolate small groups of similar mutagenic compounds within small fractions of the original sample extract. Measurements reported relative to the EOC content of the composite provide a direct connection from the human cell bioassays back to the ambient carbonaceous aerosol concentrations, which amounted to 9.15 μg m^{-3} of fine particle organic carbon averaged over the four sites studied during 1993 when each bimonthly composite sample that forms a part of the annual composite is weighted equally or 8.89 μg m^{-3} of fine particle EOC if each individual filter within the annual composite is weighted equally. These ambient concentrations have been corrected from previously published values (*5, 33*) to reflect recalibration of the air flow rate through the samples; previous results stated in terms of mutagenicity per unit organic carbon collected remain unchanged.

Extract Preparation. A brief description of sample extraction and concentration procedures appears below; for a more detailed description, see Hannigan et al. (*35*). All fine particulate matter filters used for this study were extracted in a Soxhlet apparatus with dichloromethane (DCM) for at least 16 h. These DCM extracts were then concentrated in a vacuum centrifuge down to a volume of approximately 1 mL. Extracts from all filter portions were then pooled to create a single extract. Sample portions designated for the human cell assay were exchanged into dimethyl sulfoxide

(DMSO) by adding DMSO to the DCM extract and then blowing a gentle stream of high-purity N_2 over the extract until the volume was reduced to the volume of DMSO added. Sample portions designated for chemical fractionation were reduced to a volume of just less than 100 μL by evaporation under a gentle stream of high-purity N_2. To enable comparison between our measure of organic carbon (EOC) as determined by thermal evolution and combustion analysis prior to sample extraction and the traditional measures of extracted mass as an indication of the quantity of organic compounds present, the extracted mass from a portion of this sample was measured both by the thermal evolution and combustion method and by a microscale evaporation method (*36*). The results of this test showed 0.93 μg of extracted mass/μg of EOC.

Human Cell Mutation Assay. The use of the h1A1v2 cell line for mutagenicity testing at the thymidine kinase (*tk*) locus has been described in detail elsewhere (*29*, *31*). Testing of aliquots of sample DMSO extract was performed by exposing duplicate 12-mL cultures of 1.8×10^6 exponentially growing cells for 72 h. Exposure was terminated by centrifuging and resuspending the cells in fresh media (30 mL). One day after termination of the exposure, the cultures were counted and diluted to 80 mL at 2×10^5 cells mL^{-1}. After the 3-day phenotypic expression period, cultures were plated in 96-well microtiter plates in the presence of the selective agent to determine mutagenicity ($n = 3$ with 20 000 cells per well) and in the absence of the selective agent to determine plating efficiency ($n = 2$ with 2 cells per well). Trifluorothymidine is the selective agent used for this forward mutation assay. After an additional 13-day incubation period, the plates were scored for the presence of a colony in each well. The positive control was 1.0 μg mL^{-1} benzo[*a*]pyrene, and DMSO was used as the negative control.

Plating efficiencies, mutant fractions, and their associated confidence intervals (standard deviations) were calculated using methods developed by Furth et al. (*37*). The whole sample extract and each sample fraction was tested in two independent assays to ensure test reproducibility. The results from these experiments were converted to induced mutant fraction (IMF) by subtracting the mean mutant fraction of the concurrent negative control from the mean mutant fraction observed for the filter sample extracts. Then the results of the independent assays performed on each sample were pooled to allow for quantitative evaluation of the mutagenicity of each sample fraction. The mutagenicity of a sample fraction will be described in terms of its mutagenic potency, which is defined here as the IMF per unit mass of EOC present in the whole sample prior to extraction and fractionation. As fractionation proceeds, the amount of EOC present in the original sample does not change. Thus, the mutagenic potencies of each sample fraction can be compared to each other as being parts of the whole sample. The mutagenic potency of a sample was estimated by pooling all experimental points for each sample fraction and then computing the initial slope of the dose/response relationship observed at low doses using a least-squares fit to the data that was forced through the origin (because at zero dose, there is by definition zero IMF). This technique has been used with success previously (*5*) to evaluate the seasonal and spatial variation of human cell mutagenicity of the same southern California air pollution samples that make up the composite sample tested here.

Fractionation. A successful fractionation procedure should not only isolate mutagens into smaller, less complex mixtures but also must be efficient at transmitting mutagens through the separation procedures with as little loss as possible. At the same time, losses will occur during separation, and these losses have been monitored, resulting in a range of concentrations for the targeted compounds that declines as additional processing takes place. Lafleur et al. (*16*) investigated four types of chromatographic materials to determine the degree of recovery of mutagens during column chromatography. Of the four (silica, alumina, Florisil, and cyanopropyl-bonded silica), the cyanopropyl material proved to be the most efficient material for mutagen recovery. For this reason, our sample separation sequence was initiated with a normal-phase cyanopropyl-bonded (CN) HPLC fractionation technique.

The primary fractionation procedure developed for the present study is shown in detail in Figure 1. A Varian 5000 HPLC coupled to a Hewlett-Packard 8450A UV/VIS spectrophotometer was employed. The column used had a length of 25 cm and an internal diameter of 10 mm (packed with 10 μm Alltech CN material), and the guard column was a 7-μm cyano column (Brownlee newguard column). The solvent program consisted of a 20-min hold at 95% hexane and 5% dichloromethane (DCM), a 10-min ramp to 100% DCM, a 10-min hold at 100% DCM, a 10-min ramp to 100% 2-propanol, and finally a 10-min hold at 100% 2-propanol. A flow rate of 4 mL min^{-1} was maintained. As shown in Figure 1, four fractions were created initially. The elution points that divide these four fractions from each other were chosen based on standard runs, and these standards were selected in order to isolate targeted chemical classes: unsubstituted PAC in the first and second fractions, nitroPAC in the second and third fractions, moderately polar organic compounds in the third fraction, and polar organic compounds in the fourth fraction. These four fractions are designated *nonpolar 1*, which primarily includes alkanes and PAC; *nonpolar 2*, which includes high molecular weight PAC and some lower molecular weight nitroPAC such as 9-nitroanthracene; *semipolar*, which includes numerous moderately polar compounds including higher molecular weight nitroPAC, polycyclic aromatic ketones, polycyclic aromatic quinones, and some aldehydes; and *polar*, which includes some aldehydes, alcohols, and acids. Fractionation system blanks were monitored to ensure that interference with targeted potential mutagens was negligible.

Additional fractionation steps were applied to further isolate the mutagens. The additional fractionation procedure used the primary normal-phase HPLC fractionation procedure described above as a template, with some modifications. An initial fractionation step was added to remove inactive aliphatic compounds from the bioactive aromatic compounds. This initial fractionation step involved the same HPLC described above but fitted with a size exclusion column. The size exclusion column used was 50 cm in length, 1.0 cm in diameter, and packed with 500-Å JordiGel poly(divinylbenzene) material (Jordi Associates, Inc., Bellingham, MA). The mobile phase used was DCM at a flow rate of 1.5 mL min^{-1}. This size exclusion fractionation procedure has been used previously to isolate mutagens from pond sediments (*20*) and is described by Lafleur et al. (*38*). The sample was split into two fractions, designated aliphatic and aromatic (although as expected with any fractionation procedure there is some overlap as there are some aromatic compounds in the aliphatic fraction and vice versa). The cutoff point for this separation was determined by standard runs, and the cutoff was chosen to be at the elution point of 1,6-dinitropyrene, with this compound being part of the aromatics fraction. A preliminary test using this procedure found that the chosen cut point effectively isolates bacterial mutagens in the aromatics fraction. The two fractions created by separation over the size exclusion column (aromatics and aliphatics) were then subfractionated using the primary normal-phase HPLC fractionation procedure described above with modifications in the fraction cut points needed to greatly expand the number of subfractions into which the semipolar

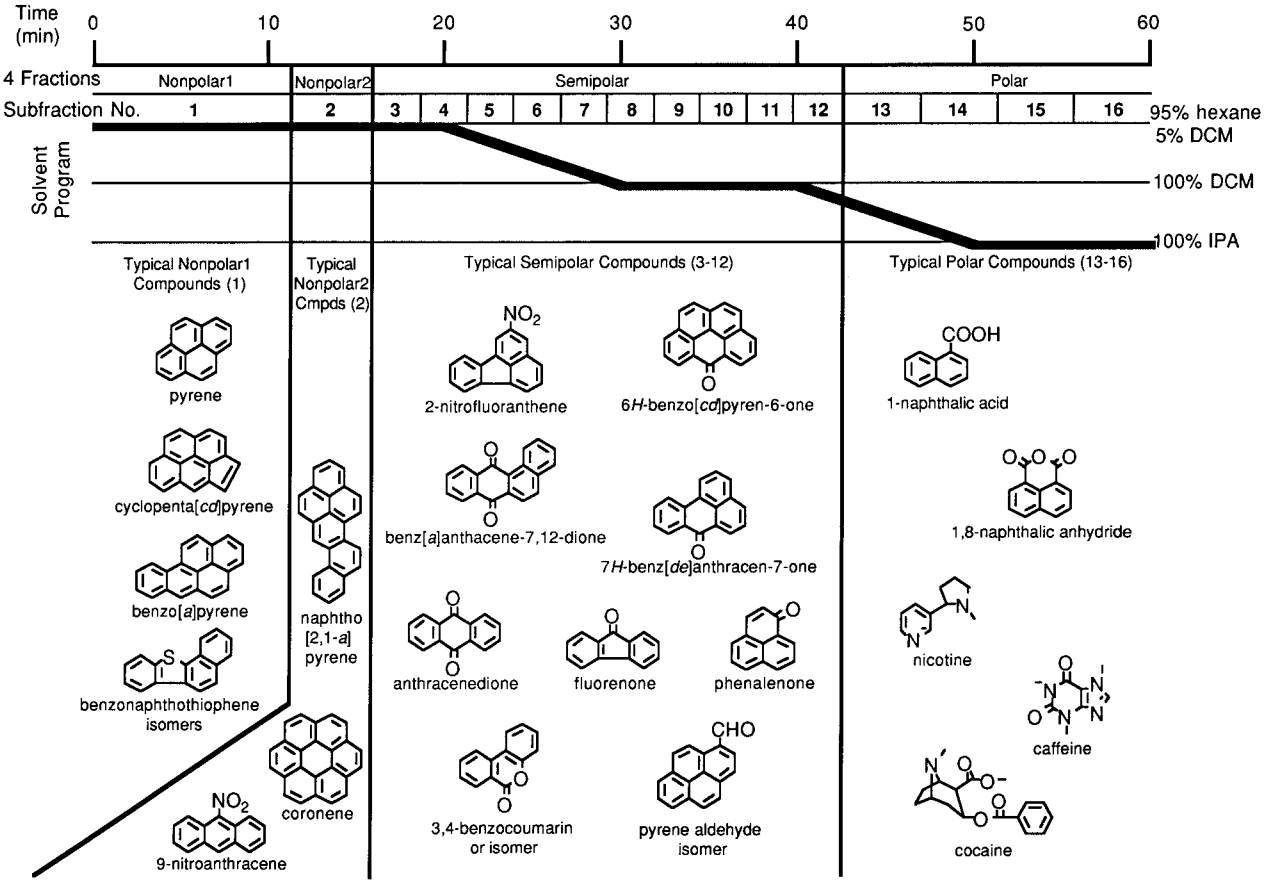

FIGURE 1. HPLC solvent program used in the normal-phase column fractionation procedure alongside the elution cut points that define the various sample fractions and subfractions studied. Also shown are the typical compounds found in each of the four fractions (see Table 1 for more examples).

and polar compounds are separated according to the flow diagrams shown in Figures 1 and 2. The aromatics fraction was divided using the same cut points as in the primary fractionation procedure for nonpolar 1 and nonpolar 2 subfractions; however, the semipolar aromatics fraction was split into 10 subfractions (designated subfractions 3a−12a), and the polar fraction was split into four subfractions (designated subfractions 13a−16a). All 19 subfractions were generated directly from the whole sample by a single size exclusion HPLC separation followed by a single normal-phase HPLC separation. Figure 2 thus does not represent reprocessing of the four-fraction level samples into 19 subfractions but rather visually represents the relationship between whole sample, fractions, and subfractions. The aliphatic nonpolar 1 and nonpolar 2 fractions were combined for further testing as neither aliphatic nonpolar fraction contained significant human cell mutagen concentrations. One purpose of removing the aliphatic material from the aromatics is to facilitate chemical analysis of the aromatics by removing much of the background petroleum alkanes from the sample, therefore making the bioactive PAC-type compounds easier to detect and measure. Authentic standards are available for dozens of unsubstituted PAC, so it is possible to quantify many PAC within a single nonpolar fraction. Very few oxyPAC are available as pure compounds for use in bioassays to identify the most important mutagens in a complex mixture of semipolar organics. The purpose of extensively subdividing the semipolar and polar extracts was to see if the mutagens in those categories could be isolated in a few small subfractions since it was unlikely that pure compounds could be obtained to aid interpretation of very complex mixtures. By extensively separating the semipolar and polar fractions, at least it can be learned whether the mutagens in these fractions are closely grouped by polarity or whether the mutagens are distributed broadly throughout these fractions.

Chemical Analysis. Analysis of mutagenic subfractions was performed using gas chromatographic separation with mass-selective detection. The system used was an HP model 5890II gas chromatograph (GC) coupled with an HP model 5972 mass-selective detector (MSD). The GC was equipped with a DB1701 fused silica capillary column (bonded 86% dimethyl−14% (cyanopropyl) phenylpolysiloxane) that had a length of 30 m, an inside diameter of 0.25 mm, and a 0.25-μm film thickness. The GC was operated as follows: split/splitless injection; constant flow of 1 mL of He min^{-1}; injector temperature of 275 °C; GC−MSD interface temperature of 275 °C; and an oven program that consisted of a 10-min hold at 65 °C, a ramp of 10 °C min^{-1} for 21-min, and a 45-min hold at 275 °C. The MSD was operated in selected ion monitoring (SIM) and full-scan (i.e., total ion monitoring) modes for the nonpolar 1, nonpolar 2, and semipolar fractions and in full-scan mode for the polar fractions.

SIM methods were developed for each of the three types of fractions studied by that approach. Each SIM method was developed by creating a list of target compounds that might appear in that fraction. Target compounds are defined as any organic compound that falls into one of the following categories: (i) compound has been tested in the h1A1v2 assay and is known to be a human cell mutagen (a list of these compounds is given by Durant et al. (*32*)); (ii) compound is a known bacterial mutagen (based on extensive literature review). The key ions of the target compounds were entered into a specific fraction's SIM method following method development work performed in full-scan mode conducted on a Long Beach winter atmospheric aerosol test sample. Key ions were selected by a combination of standard runs,

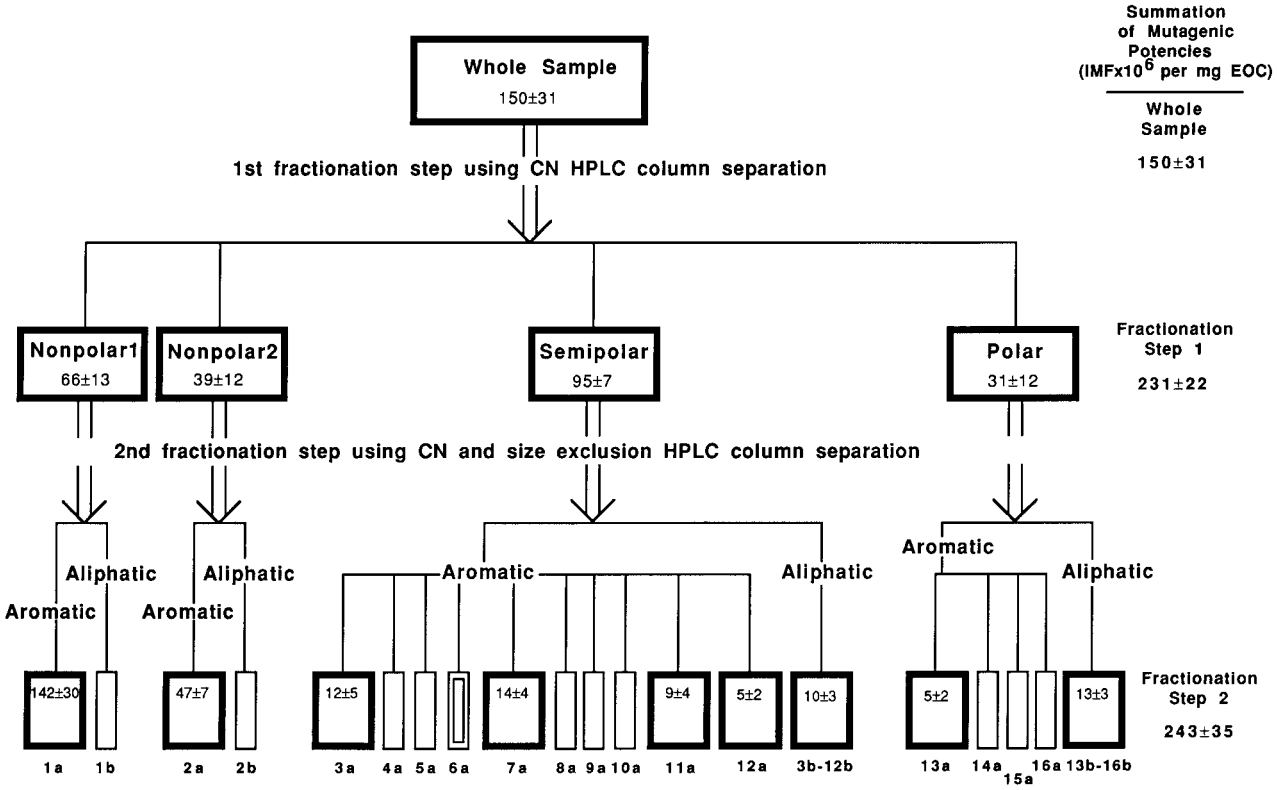

FIGURE 2. Flow chart for the bioassay-directed chemical analysis. Mutagenic fractions and subfractions are indicated in bold outline, and the mutagenic potencies (±1 SD) of those fractions and subfractions are given inside the boldly outlined boxes, units are IMF ($\times 10^6$). The subfraction that is double boxed (subfraction 6a) has a mutagenic potency of 16 ± 9 IMF ($\times 10^6$)/mg of EOC, which is not significantly different from zero in a statistical sense, but the best estimate of the mutagenic potency is high enough that this subfraction warrants continued investigation.

literature review if no standard existed, and in light of the observed signal-to-noise ratio for those key ions observed during full-scan mode runs performed on the Long Beach winter test sample. Due to the extremely limited information on the mutagenicity of polar compounds, the development of a SIM method that targeted single compounds that were suspected in advance to be mutagenic within the polar fractions was not pursued. Instead the polar fractions were searched in the full-scan mode to identify the prominent compound peaks that were present. Polar fractions were run under two different conditions: (i) as is and (ii) derivatized through the addition of diazomethane to convert labile organic acids to their methyl ester analogues.

Compounds were identified by comparison with authentic standards where available, with the Wiley (5th edition) and the NIST mass spectral library, and with published mass spectra from the literature. Quantification was achieved through standard runs. A relative response factor (RRF) that relates the compound key ion area counts to the compound mass through the use of a co-injection standard (1-phenyldodecane) was developed for each compound through injection of standards. Certain standards exhibited different RRF for low concentration versus high concentration analyses, and for these compounds a second (low concentration) RRF was determined. Compound identification and quantification were categorized as follows: (a) *positive*, when the sample spectrum and retention time matches that of an authentic standard; (b) *probable*, when sample spectrum matches the NIST mass spectral library, sample relative retention time matches published values, and RRF for a compound with a similar molecular weight (MW) and functional group was used; (c) *possible*, when sample spectrum matches the NIST mass spectral library or sample spectrum and relative retention time matches published values, and RRF for a compound with similar structure was used; (d) *tentative*, when sample spectrum contains additional mass fragments from one or more coeluting compounds (noise) as compared to the NIST mass spectral library and/or published values, and RRF for a compound with similar structure was used. A mixture of standards was run multiple times during the sample runs to check GC/MS performance.

Human Cell Assay Results

Unfractionated (Whole) Sample Extract. The dose–response curve generated from the bioassays of the unfractionated (whole) annual composite sample as well as the least squares linear fit used to obtain a mutagenic potency value for the whole sample are shown in Figure 3. The resulting mutagenic potency (i.e., induced mutant fraction per mass of fine particulate organic carbon present in the ambient sample composite prior to extraction) was determined to be 150 ± 31 IMF ($\times 10^6$)/mg of EOC. In a previous study (5) in which the human cell mutagenicity of bimonthly composites of the same air pollution samples analyzed separately at each urban site was investigated, annual average mutagenic potency values (IMF ($\times 10^6$)/mg of EOC) were calculated for each site: Long Beach, 176; central Los Angeles, 140; Azusa, 145; and Rubidoux, 137. The similarity of the mutagenic potency of the annual urban composite extract used for the present study versus the arithmetic average of the bimonthly composites is an indication that the earlier assay results are reproducible.

Fractionated Sample Extracts. The dose–response curves along with the least squares linear fit used to obtain mutagenic potency values for each sample at the four-fraction level are shown in Figure 3. The mutagenic potency values determined from this experiment appear in Figure 2. Fractions having a mutagenic potency significantly greater than

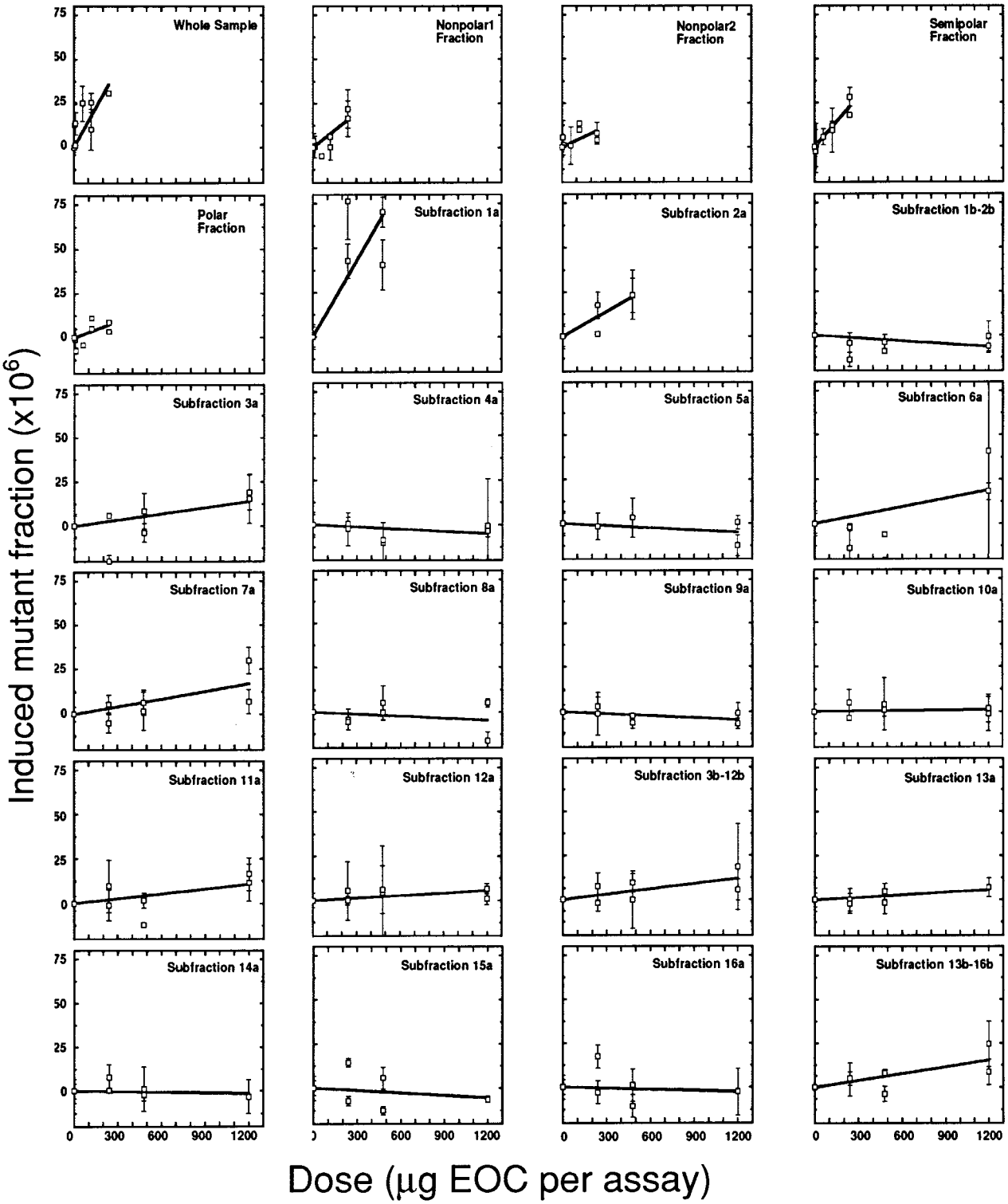

FIGURE 3. Dose−response plots for the whole sample extract, each of the four fractions created at the first separation step, and each of the subfractions created at the second fractionation step. Multiple points at a given dose represent independent experiments at that dose, and error bars represent ±1 SD about the experimental mean. Least-squares linear fit to the data (forced through the origin) is shown for each sample.

zero are presented in bold outline in that figure, and the mutagenic potency values for the active fractions are given inside each boldly outlined box in units of IMF ($\times 10^6$)/mg of EOC in the whole sample originally supplied to the extraction and fractionation process. At the first four-way separation step, all fractions exhibit some mutagenicity with nonpolar 1 producing a potency of 66 ± 13 IMF ($\times 10^6$)/mg of EOC, nonpolar 2 accounting for a potency of 39 ± 12 IMF ($\times 10^6$)/mg of EOC, the semipolar fraction contributing a potency increment of 95 ± 7 IMF ($\times 10^6$)/mg of EOC, and the polar fraction accounting for a potency increment of 31 ± 12 IMF ($\times 10^6$)/mg of EOC. The sum of the mutagenic potency increments from the four fractions totals 231 ± 22 IMF ($\times 10^6$)/mg of EOC or 138% of that of the unfractionated sample extract. Such a gain in mutagenic potency upon fractionation has been observed in other studies (*20*) and could well be

due to reduced interference among compounds when the bioassays are performed on the less complex mixtures present in the fractionated samples. This fractionation procedure isolated most of the mutagenicity into two fractions, nonpolar 1 and semipolar. The nonpolar 1 fraction, besides containing PAC, also contains numerous other nonpolar compounds, most aliphatic in nature, and is thus still an extremely complex mixture. The semipolar fraction, as seen in Figure 1, contains a wide range of compound classes, and isolating the activity into this fraction gives little clue as to mutagen identity. Therefore, refinement of the fractionation procedure was undertaken.

Subfractionated Sample Extracts. The annual urban composite extract was further separated into the many subfractions described in Figures 2 and 3 in order to further isolate the important mutagens from each other. The absolute mutagenic potency values of the two extracts tested (whole aliphatic and whole aromatic) summed to 163 ± 34 IMF ($\times 10^6$)/mg of EOC, which is statistically indistinguishable at the 95% confidence level from the 150 ± 31 IMF ($\times 10^6$)/mg of EOC value determined for the original unfractionated whole sample extract. The whole aromatics fraction contained 83% of the mutagenic potency, and the whole aliphatics fraction contained 17% of the mutagenic potency. Use of this procedure based on the size exclusion column confirms as expected that most of the mutagenicity exists within the aromatics fraction.

These two fractions, aromatics and aliphatics, were then further separated using the same normal-phase HPLC procedure used to create the four fractions (nonpolar 1, nonpolar 2, semipolar, polar) discussed previously. The aromatics extract was split such that 10 additional subfractions were created within the semipolar group and four additional subfractions were created within the polar group. The elution times at which the subfractions are divided are shown near the top of Figure 1; i.e., subfractions 1−16. The aliphatics extract was split into the four subfractions defined earlier: aliphatic nonpolar 1, aliphatic nonpolar 2, aliphatic semipolar, and aliphatic polar. All subfractions were tested for mutagenicity at the *tk* locus of h1A1v2 cells using a 72-h exposure in two independent experiments; the aliphatics nonpolar 1 and 2 were combined during testing as no mutagens were found in either fraction. Low-extract dose levels were used that were similar to those used for testing of the unfractionated extract, and additional high-dose bioassays were added in order to see smaller increments of mutagenic potency that might be contained within the least potent extract subfractions.

The results of these bioassays are shown in Figure 2. The mutagenicity seen in the nonpolar fractions has been isolated into the aromatic subfractions. The mutagenic potency value for the aromatic nonpolar 1 subfraction is significantly larger with greater than 95% confidence than was observed in the nonpolar 1 fraction; 142 ± 30 IMF ($\times 10^6$)/mg of EOC vs 66 ± 13 IMF ($\times 10^6$)/mg of EOC, while the potency of the aromatic nonpolar 2 fraction is very similar to that of the unseparated nonpolar 2 fraction (39 ± 12 versus 47 ± 7 IMF ($\times 10^6$)/mg of EOC). The mutagenicity seen in the semipolar fraction has been isolated into 4 of the 10 aromatic semipolar subfractions and in the aliphatic semipolar subfraction. If we sum the mutagenic potency values observed in these subfractions, we get a total for the semipolar subfractions of 50 ± 13 IMF ($\times 10^6$)/mg of EOC. This is a decrease from what was observed in the semipolar fraction, which exhibited a mutagenic potency value of 95 ± 7 IMF ($\times 10^6$)/mg of EOC before it was subdivided. The mutagenicity observed in the polar fraction has been isolated into one of the four aromatic polar subfractions and in the aliphatic polar subfraction. The sum of the absolute values of the subfraction mutagenic potency values totals 243 ± 35 IMF ($\times 10^6$)/mg of EOC or 162% of that of the unfractionated whole sample extract and 105% of that of the total of the mutagenic potencies at the four-fraction level. This subfractionation procedure successfully isolated most of the overall sample mutagenicity within a few subfractions that are chemically less complex and then the total sample. The results of the targeted chemical analysis of suspected mutagens in the subfractions next will be given for those subfractions that exhibited significant mutagenic potency.

Chemical Analysis Results

Aromatics Nonpolar 1. The organic compounds quantified in this subfraction are shown in Table 1. Mass concentrations are shown in units of nanograms of a specific compound per milligram of EOC (organic carbon on the atmospheric filter samples as determined by thermal evolution and combustion analysis prior to sample extraction). As can be seen from this table, 43 specific PAC and an additional 37 PAC that are partially characterized have been quantified in this subfraction, accounting for 1690−455 ng of PAC/mg of EOC. The range of concentrations shown in Table 1 reflects the range of concentrations observed for each compound when quantified in the whole sample extract and in the various fractionated and subfractionated extracts. Generally, the highest values are those seen in the whole extract as it has been subjected to the least opportunity for losses during separations and processing. PAC and *n*-alkanes were quantified in SIM mode. Other compounds identified in the full-scan mode in this subfraction include cholestanes, hopanes, and benzonaphthothiophene isomers intended for use as source tracers (*39*) that will be described in a later paper on that subject.

Also shown in Table 1 is the range of estimated mutagenic potency contributions from specific compounds. The mutagenic potency contribution values are calculated by multiplying the mass concentration range of a specific compound (ng/mg of EOC) by the mutagenic potency (IMF ($\times 10^6$)/ng) value for that specific compound if it is known from prior bioassays applied to that pure compound (*32*). If a "*u*" (i.e., unknown) appears in this column, then this compound has not been tested in the h1A1v2 assay; therefore, no mutagenic potency value can be calculated. The overall mutagenic potency of the aromatics in the nonpolar 1 subfraction is 66 ± 13−142 ± 30 IMF ($\times 10^6$)/mg of EOC based on the results shown in Figure 2. The sum of the mutagenic potency contributions of the 31 compounds identified in this subfraction for which pure compound bioassays have been conducted to date is 25.1−7.7 IMF ($\times 10^6$)/mg of EOC, roughly 38−5% of this subfraction's total activity. The largest single contributor to the activity among known compounds is due to cyclopenta[*cd*]pyrene, which accounts for 12.9/25.1−5.3/7.7 = 51−69% of the activity that can be assigned to a specific compound. Other contributing compounds include benzo[*a*]pyrene at 3.72−0.87 IMF ($\times 10^6$)/mg of EOC, benzo[*ghi*]perylene at 2.43−0.36 IMF ($\times 10^6$)/mg of EOC, benzo[*b*]fluoranthene at 2.06−0.51 IMF ($\times 10^6$)/mg of EOC, indeno[1,2,3-*cd*]pyrene at 1.73−0.24 IMF ($\times 10^6$)/mg of EOC, and benzo[*k*]fluoranthene at 1.17−0.26 IMF ($\times 10^6$)/mg of EOC.

Ten additional PAC that can be named specifically remain untested in the h1A1v2 assay; their contribution to the overall mutagenicity of this fraction can be computed in the future if such bioassays are performed. Table 1 shows that numerous heavy PAC isomers exist in the ambient sample composite that have not been identified specifically because standards do not exist. These heavy PAC are potentially important mutagens as is evidenced by the fact that three of the four most mutagenic compounds tested in the h1A1v2 assay are 302μ PAC (i.e., PAC with molecular weight of 302 amu) (*32*). Further notice that quantifiable amounts of

TABLE 1. Organic Compounds Quantified in the Mutagenic Subfractions

compound	formula	concn range (ng/mg of EOC)[a]	single compd mutagenic potency (IMF ($\times 10^6$)/ng)[b]	contribution to potency of ambient mixture (IMF ($\times 10^6$)/mg of EOC)	ID[c]
Aromatic Nonpolar 1 Subfraction 1a					
polycyclic aromatic hydrocarbons (PAH)					
phenanthrene	$C_{14}H_{10}$	24.3−13.9	0.00	0.00	a
anthracene	$C_{14}H_{10}$	1.6−0.8	u	u	a
3-methylphenanthrene	$C_{15}H_{12}$	8.5−3.9	u	u	b
2-methylphenanthrene	$C_{15}H_{12}$	10.4−5.8	u	u	b
2-methylanthracene	$C_{15}H_{12}$	1.5−0.6	u	u	a
9-methylphenanthrene	$C_{15}H_{12}$	4.5−2.3	u	u	b
1-methylphenanthrene	$C_{15}H_{12}$	3.7−2.6	1.5×10^{-4}	5.6−3.9 ($\times 10^{-4}$)	a
total methyl 178 μ PAH	$C_{15}H_{12}$	31.3−17.3			
total C_2H_6 178 μ PAH	$C_{16}H_{14}$	63.0−24.4			
retene	$C_{18}H_{18}$	10.0−6.3	u	u	a
fluoranthene	$C_{16}H_{10}$	45.0−21.4	0.00	0.00	a
acephenanthrylene	$C_{16}H_{10}$	6.7−5.3	u	u	b
pyrene	$C_{16}H_{10}$	52.5−25.8	0.00	0.00	a
2-methylfluoranthene	$C_{17}H_{12}$	4.9−3.2	u	u	a
total methyl 202 μ PAH	$C_{17}H_{12}$	48.7−24.3			b
total C_2H_6 202 μ PAH	$C_{18}H_{14}$	31.5−17.8			b
benzo[*a*]fluorene	$C_{17}H_{12}$	2.8−0.7	0.00	0.00	b
benzo[*b*]fluorene	$C_{17}H_{12}$	6.0−3.4	0.00	0.00	a
benzo[*c*]phenanthrene	$C_{18}H_{12}$	5.7−2.8	0.0073	0.04−0.02	a
benzo[*ghi*]fluoranthene	$C_{18}H_{10}$	34.7−16.2	0.00	0.00	b
cyclopent[*hi*]acephenanthrylene	$C_{18}H_{10}$	1.2[d]	u	u	b
cyclopent[*hi*]aceanthrylene	$C_{18}H_{10}$	ND	u	u	b
benz[*a*]anthracene	$C_{18}H_{12}$	23.9−12.2	4.0×10^{-4}	9.6−4.8 ($\times 10^{-3}$)	a
cyclopenta[*cd*]pyrene	$C_{18}H_{10}$	20.3−8.3	0.64	12.93−5.31	a
chrysene & triphenylene	$C_{18}H_{12}$	47.2−24.8	4.0×10^{-4}	0.02−0.01	a
total methyl 226 μ PAH	$C_{19}H_{12}$	34.9−7.6			b
total methyl 228 μ PAH	$C_{19}H_{14}$	80.6−25.8			b
total C_2H_6 228 μ PAH	$C_{19}H_{16}$	38.7−7.2			b
benzo[*k*]fluoranthene	$C_{20}H_{12}$	76.7−17.0	0.015	1.17−0.26	a
benzo[*b*]fluoranthene	$C_{20}H_{12}$	71.5−17.7	0.029	2.06−0.51	a
benzo[*j*]fluoranthene	$C_{20}H_{12}$	15.3−3.9	0.013	0.20−0.05	a
benzo[*e*]pyrene	$C_{20}H_{12}$	84.5−17.6[e]	2.1×10^{-4}	18−3.7 ($\times 10^{-3}$)	a
benzo[*a*]pyrene	$C_{20}H_{12}$	33.9−7.9	0.11	3.72−0.87	a
perylene	$C_{20}H_{12}$	21.6−4.8	1.3×10^{-4}	2.9−0.6 ($\times 10^{-3}$)	a
total methyl 252 μ PAH	$C_{21}H_{14}$	237−34.3			b
276 μ PAH isomer	$C_{22}H_{12}$	29.6−3.5	u	u	b
276 μ PAH isomer	$C_{22}H_{12}$	1.3[d]	u	u	b
276 μ PAH isomer	$C_{22}H_{12}$	32.1−4.2	u	u	b
276 μ PAH isomer	$C_{22}H_{12}$	3.3[d]	u	u	b
indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	87.2−11.8[e]	0.020	1.73−0.24	a
276 μ PAH isomer	$C_{22}H_{12}$	1.3[d]	u	u	b
benzo[*ghi*]perylene	$C_{22}H_{12}$	190−27.9[e]	0.013	2.43−0.36	a
276 μ PAH isomer	$C_{22}H_{12}$	0.8[d]	u	u	b
anthanthrene	$C_{22}H_{12}$	6.0[d]	u	u	a
278 μ PAH isomer	$C_{22}H_{14}$	1.5[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	2.1[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	2.0[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	3.0[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	2.9[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	2.2[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	0.6[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	2.2[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	1.7[d]	u	u	b
278 μ PAH isomer	$C_{22}H_{14}$	2.9[d]	u	u	b
dibenz[*a,h*]anthracene	$C_{22}H_{14}$	41.9−3.7	0.020	0.82−0.07	a
benzo[*b*]chrysene	$C_{22}H_{14}$	9.4[d]	u	u	a
278 μ PAH isomer	$C_{22}H_{14}$	13.6−1.0	u	u	b
picene	$C_{22}H_{14}$	21.5−3.6	1.4×10^{-4}	3.0−0.5 ($\times 10^{-3}$)	a
300 μ PAH isomer	$C_{24}H_{12}$	1.0[d]	u	u	b
300 μ PAH isomer	$C_{24}H_{12}$	0.6[d]	u	u	b
300 μ PAH isomer	$C_{24}H_{12}$	3.7[d]	u	u	b
300 μ PAH isomer	$C_{24}H_{12}$	1.1[d]	u	u	b
coronene	$C_{24}H_{12}$	28.8[d]	1.5×10^{-4}	4.3×10^{-3}	a
302 μ PAH isomer	$C_{24}H_{14}$	0.2[d]	u	u	b
302 μ PAH isomer	$C_{24}H_{14}$	0.1[d]	u	u	b
302 μ PAH isomer	$C_{24}H_{14}$	0.1[d]	u	u	b
302 μ PAH isomer	$C_{24}H_{14}$	0.3[d]	u	u	b
302 μ PAH isomer	$C_{24}H_{14}$	0.2[d]	u	u	b
302 μ PAH isomer	$C_{24}H_{14}$	0.5[d]	u	u	b
302 μ PAH isomer	$C_{24}H_{14}$	0.5[d]	u	u	b

TABLE 1 (Continued)

compound	formula	concn range (ng/mg of EOC)[a]	single compd mutagenic potency (IMF ($\times 10^6$)/ng)[b]	contribution to potency of ambient mixture (IMF ($\times 10^6$)/mg of EOC)	ID[c]
Aromatic Nonpolar 1 Subfraction 1a					
naphtho[2,3-*j*]fluoranthene	$C_{24}H_{14}$	0.7[d]	6.5×10^{-4}	4.6×10^{-4}	a
naphtho[1,2-*k*]fluoranthene	$C_{24}H_{14}$	0.7[d]	0.0081	0.01	a
naphtho[2,3-*b*]fluoranthene	$C_{24}H_{14}$	ND	0.0083	0.00	a
dibenzo[*b,k*]fluoranthene	$C_{24}H_{14}$	ND	0.020	0.00	a
dibenzo[*a,l*]pyrene	$C_{24}H_{14}$	ND	1.73	0.00	a
dibenzo[*a,k*]fluoranthene	$C_{24}H_{14}$	0.4[d]	0.023	0.01	a
naphtho[2,3-*k*]fluoranthene	$C_{24}H_{14}$	0.3[d]	3.4×10^{-4}	1.0×10^{-4}	a
dibenzo[*a,e*]pyrene	$C_{24}H_{14}$	ND	0.24	0.00	a
naphtho[2,1-*a*]pyrene	$C_{24}H_{14}$	ND	0.024	0.00	a
total 302 μ PAH	$C_{24}H_{14}$	4.0[d]			
total PAH		1690−455		25.1−7.72	
Aromatic Nonpolar 2 Subfraction 2a					
PAH					
benzo[*e*]pyrene	$C_{20}H_{12}$	0.5−0.1[e]	2.1×10^{-4}	1.0−0.2 ($\times 10^{-4}$)	a
indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	0.7−0.1[e]	0.020	0.01−0.00	a
benzo[*ghi*]perylene	$C_{22}H_{12}$	3.4−0.5[e]	0.013	0.04−0.01	a
coronene	$C_{24}H_{12}$	2.8[d]	1.5×10^{-4}	4.2×10^{-4}	a
naphtho[2,3-*b*]fluoranthene	$C_{24}H_{14}$	1.4[d]	0.0083	0.01	a
dibenzo[*b,k*]fluoranthene	$C_{24}H_{14}$	1.4[d]	0.020	0.03	a
dibenzo[*a,k*]fluoranthene	$C_{24}H_{14}$	1.2[d]	0.023	0.03	a
naphtho[2,3-*k*]fluoranthene	$C_{24}H_{14}$	1.2[d]	3.4×10^{-4}	4.1×10^{-4}	a
dibenzo[*a,e*]pyrene	$C_{24}H_{14}$	0.6[d]	0.24	0.14	a
naphtho[2,1-*a*]pyrene	$C_{24}H_{14}$	0.4[d]	0.024	0.01	a
total PAH		13.6−9.7		0.27−0.23	
nitro-PAH					
9-nitroanthracene	$C_{14}H_9NO_2$	3.8[d]	3.9×10^{-4}	1.5×10^{-3}	a
Aromatic Semipolar Subfraction 3a					
polycyclic aromatic ketones (PAK) and quinones (PAQ)					
fluorenone	$C_{13}H_8O$	49.2−10.5[e]	u	u	a
phenanthrenone or isomer	$C_{14}H_{10}O$	3.2[d]	u	u	c
4*H*-cyclopenta[*def*]phenanthren-4-one	$C_{15}H_8O$	10.6−3.8[e]	0.00	0.00	a
1-methylanthracene-9,10-dione	$C_{15}H_{10}O_2$	11.7−2.4	u	u	b
dimethylanthracene-9,10-dione	$C_{16}H_{12}O_2$	5.6[d]	u	u	c
11*H*-benzo[*a*]fluoren-11-one	$C_{17}H_{10}O$	30.4−16.2[e]	u	u	b
methylbenzathrone or isomer	$C_{18}H_{12}O$	13.1[d]	u	u	c
benz[*a*]anthracen-7,12-dione	$C_{18}H_{10}O_2$	38.2−13.4[e]	0.00	0.00	a
total PAK and PAQ		162−68.2		0.00	
nitro-PAH					
2-nitrofluoranthene	$C_{16}H_9NO_2$	28.4−6.4	0.019	1.27−0.12	a
1-nitropyrene	$C_{16}H_9NO_2$	0.2[d]	0.0013	2.6×10^{-4}	a
2-nitropyrene	$C_{16}H_9NO_2$	2.3[d]	0.00	0.00	b
total nitro-PAH		30.9−8.9		1.27−0.12	
Aromatic Semipolar Subfraction 6a					
PAK and PAQ					
7*H*-benz[*de*]anthracen-7-one	$C_{17}H_{10}O$	81.3−49.9[e]	4.0×10^{-4}	0.03−0.02	a
11*H*-benzo[*b*]fluoren-11-one	$C_{17}H_{10}O$	3.5−2.0[e]	u	u	b
methylbenzanthrone or isomer	$C_{18}H_{12}O$	18.8[d]	u	u	c
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$	90.7−44.5[e]	0.018	1.63−0.80	a
naphthacene-5,12-dione	$C_{18}H_{10}O_2$	13.2−4.8	0.00	0.00	b
total PAK and PAQ		208−120		1.66−0.82	
other polycyclic aromatic carbonyls (PAC)					
pyrene aldehyde or isomers	$C_{16}H_{10}O$	10.0−9.7[e]	u	u	b
3,4-benzocoumarin or isomer	$C_{13}H_8O_2$	25.3−9.7[e]	u	u	b
3,4,4a,5-naphthocoumarin or isomer	$C_{15}H_8O_2$	15.8−11.1[e]	u	u	b
3,4-naphthocoumarin or isomer	$C_{17}H_{10}O_2$	2.4[d]	u	u	c
xanthone	$C_{13}H_8O_2$	8.2−1.8	u	u	a
phthalic anhydride	$C_8H_4O_3$	5.9[d]	u	u	c
total PAC		67.6−40.6		0.00	
Aromatic Semipolar Subfraction 7a					
PAK and PAQ					
7*H*-benz[*de*]anthracen-7-one	$C_{17}H_{10}O$	1.1−0.7[e]	4.0×10^{-4}	4.4−2.8 ($\times 10^{-4}$)	a
cyclopenta[*cd*]pyren-3(4*H*)-one	$C_{18}H_{10}O$	2.4−2.2	6.6×10^{-4}	1.6−1.5 ($\times 10^{-3}$)	a
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$	39.7−19.5[e]	0.018	0.72−0.35	a
cyclopenta[*cd*]pyrenedione or isomer	$C_{18}H_8O_2$	5.4[d]	u	u	c
total PAK and PAQ		48.6−27.8		0.72−0.35	

TABLE 1 (Continued)

compound	formula	concn range (ng/mg of EOC)[a]	single compd mutagenic potency (IMF ($\times 10^6$)/ng)[b]	contribution to potency of ambient mixture (IMF ($\times 10^6$)/mg of EOC)	ID[c]
Aromatic Semipolar Subfraction 7a					
PAC					
3,4-naphthocoumarin or isomer	$C_{17}H_{10}O_2$	3.1[d]	u	u	c
dimethylphthalide isomers	$C_{10}H_{10}O_2$	6.8[d]	u	u	d
methylphthalide isomers	$C_9H_8O_2$	3.1[d]	u	u	d
nitro-PAC					
hydroxynitrofluorenone isomer	$C_{13}H_7NO_4$	2.8[d]	u	u	d
Aromatic Semipolar Subfractions 11a and 12a					
PAC					
1,8-naphthalic anhydride	$C_{12}H_6O_3$	1.1[d]	0.00	0.00	a
1,8-naphthalic amide	$C_{12}H_7NO_2$	5.0[d]	u	u	c
2,3-naphthalic amide	$C_{12}H_7NO_2$	1.8[d]	u	u	c
phthalamide isomer	$C_8H_5NO_2$	45.9[d]	u	u	c
dimethylazanaphthalene isomers	$C_{11}H_{11}N$	109[d]	u	u	d
azanaphthalenedione isomer	$C_9H_7NO_2$	15.6[d]	u	u	d
total PAC		178		0.00	
nitro-PAC					
nitrobenzocoumarin isomer	$C_{13}H_7NO_4$	3.3[d]	u	u	d
nitroindole isomer	$C_8H_6N_2O_2$	78.2[d]	u	u	d
total nitro-PAC		81.5		0.00	
Aliphatic Semipolar Subfraction 3b−12b					
PAC					
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$	1.6−0.8[e]	0.018	0.03−0.01	a
cyclopenta[*ghi*]perylenone or isomer	$C_{21}H_{10}O$	1.3[d]	u	u	b
1,8-naphthalic anhydride	$C_{12}H_6O_3$	5.0[d]	0.00	0.00	a
total PAC		7.9−7.1		0.03−0.01	
Aromatic Polar Subfraction 13a					
PAC					
1,8-naphthalic anhydride	$C_{12}H_6O_3$	86.7[d]	0.00	0.00	a
1,2-naphthalic anhydride	$C_{12}H_6O_3$	3.1[d]	0.00	0.00	a
1,10-phenanthrene dicarboxylic acid anhydride or isomer	$C_{16}H_8O_3$	7.5[d]	u	u	b
naphthoic acid isomer	$C_{11}H_8O_2$	17.6[d]	u	u	b
vanillin	$C_8H_8O_3$	14.6[d]	u	u	d
total PAC		130		0.00	
other compounds					
nonanal	$C_9H_{18}O$	164−72.7[e]	u	u	a
decanal	$C_{10}H_{20}O$	21.5[d]	u	u	b
caffeine	$C_8H_{10}N_4O_2$	25.4[d]	u	u	a
cocaine	$C_{17}H_{21}NO_4$	11.4−5.2[e]	u	u	a
total		222−125		0.00	
Aliphatic Polar Subfractions 13b−16b					
PAC					
1,8-naphthalic anhydride	$C_{12}H_6O_3$	1.4[d]	0.00	0.00	a
1,2-naphthalic anhydride	$C_{12}H_6O_3$	0.5[d]	0.00	0.00	a
total PAC		1.9		0.00	
other compounds					
nonanal	$C_9H_{18}O$	83.4−36.9[e]	u	u	a
decanal	$C_{10}H_{20}O$	9.4[d]	u	u	b
nicotine	$C_{10}H_{14}N_2$	12.9[d]	u	u	a
caffeine	$C_8H_{10}N_4O_2$	4.6[d]	u	u	a
cocaine	$C_{17}H_{21}NO_4$	8.3−3.8[e]	u	u	a
total		119−67.6		0.00	

[a] Range of concentrations shown reflect the range of concentrations measured for each compound when quantified in the whole sample extract and in the various subfractionated extracts. Generally, the highest values are those seen in the whole extract as it has been subjected to the least opportunity for losses during separations and processing. Compound concentrations are stated relative to the quantity obtained when processing 1 mg of organic carbon (EOC) contained within the whole ambient composite filter samples prior to extraction. The Los Angeles area 1993 ambient annual average composite airborne fine particle organic carbon concentration was 8.89 μg/m^3 of EOC. [b] Single compound mutagenic potency in the h1A1v2 assay as determined by regression analysis applied to the data collected by Durant and co-workers (*32*). u is given for compounds that have not yet been tested in that assay, and therefore the mutagenic potency is unknown. Units are induced mutant fraction multiplied by 10^6/ng of compound applied to the standard 12 mL h1A1v2 assay. ND, not detected. [c] Compounds have been identified and quantified at different levels of confidence; a = positive, b = probable, c = possible, d = tentative (see criteria in text). [d] Compound measurable only in subfractions; not measured in whole sample. [e] Compound also measured in another subfraction, so the concentration reported as quantified from the whole sample extract was determined by multiplying the total concentration quantified in the whole extract by the percentage of the compound concentration occurring in this specific subfraction.

methyl PAC isomers also exist in this sample subfraction. These methyl isomers are potentially important mutagens as all of the methyl isomers tested at present in the h1A1v2 assay are more potent than the parent PAC (*32*). Thus, conceivably, the methyl PAC isomers and the unidentified heavy (5+ rings) PAC may account for the remaining portion of this subfraction's activity. It is worth noting that a single PAC, cyclopenta[*cd*]pyrene, accounts for most of the mutagenic activity identified in this sample to date (potentially up to 12.9 of the 150 IMF ($\times 10^6$)/mg of EOC found in the whole sample); it may not take more than a few additional very active compounds to account for the remaining activity of the aromatic nonpolar 1 sample subfraction.

Aromatics Nonpolar 2. The organic compounds found in this subfraction are shown in Table 1. Notice that some of the heavier PAC are contained within this subfraction. Other compounds that were sought in the SIM mode include a full suite of nitro-PAC, aromatic ketones and quinones; a detailed list appears in Table 2. Of these compounds only 9-nitroanthracene was identified in this subfraction. Thirteen other peaks are visible in the full-scan mode, and from these peaks, quinoline and squalene were identified as being present.

The mutagenic potency of the aromatics in this subfraction is in the range $39 \pm 12 - 47 \pm 7$ IMF ($\times 10^6$)/mg of EOC based on the results shown in Figure 2. The sum of the mutagenic potency contributions of the compounds identified in this subfraction is 0.27−0.23 IMF ($\times 10^6$)/mg of EOC. Thus, an insignificant amount of this subfraction's activity can be accounted for by those compounds identified in this study. Again, the further identification of the heavy PAC isomers could be an important next step. The single nitro-PAC identified accounted for essentially none of the activity.

Aromatic Semipolar Subfractions. The organic compounds identified in the four aromatic semipolar subfractions that contain significant mutagenic activity are shown in Table 1. In addition, chemical analysis of another aromatic semipolar subfraction (6a), which exhibited a high nominal mutagenic potency value (16 IMF ($\times 10^6$)/mg of EOC) but with a large uncertainty (± 9 IMF ($\times 10^6$)/mg of EOC), also appears in Table 1 and will be discussed. The compounds that were on the list of targeted semipolar organics that were sought by the SIM method but that were not found in a mutagenic subfraction appear in Table 2. This list includes numerous oxygenated and nitrated aromatic compounds.

The targeted compounds identified in subfraction 3a consist primarily of aromatic ketones, aromatic quinones, and nitro aromatics. This subfraction was also processed in full-scan mode, and 10 additional peaks were found (i.e., 10 more peaks than were quantified using the list of targeted compounds that were sought in SIM mode). These 10 peaks include the following compounds: benzanthrone or isomer, dimethyl anthracene-9,10-dione, methylbenzanthrones or isomers, and four partially aromatic ketones. These additional compounds are entered in Table 1 with the exception of the four partially aromatic ketones for which we have no corresponding similar compounds from which to estimate a response factor. This subfraction's mutagenic potency is 12 ± 5 IMF ($\times 10^6$)/mg of EOC. The lone significant contributing compound identified in this subfraction is 2-nitrofluoranthene, which accounts for 1.27−0.12 IMF ($\times 10^6$)/mg of EOC. Additional testing of pure compounds is recommended.

Subfraction 6a consists mostly of oxygenated aromatic compounds, including ketones, quinones, coumarins, and aldehydes. A full-scan mode analysis of this sample found 23 additional peaks in this subfraction, of which 10 can be tentatively identified as methylbenzanthrones or isomers, 3,4-naphthocoumarin or isomer, phthalic anhydride, diphenyl methyl pentene isomers, and 2,4-bis(dimethyl)-6-*tert*-butylphenol. Those identified aromatic compounds found by full-scan mode analysis have been entered in Table 1 as well. This subfraction's mutagenic potency is 16 ± 9 IMF

TABLE 2. Targeted Semipolar Compounds Sought in SIM Mode That Are Either Found in Subfractions Other Than the Mutagenic Subfractions Shown in Table 1 or That Are Not Found at All

compound	concn range (ng/mg of EOC)[a]
Targeted Compounds Found in Nonmutagenic Subfractions	
Aromatic Semipolar Subfraction 4a	
anthracene-9,10-dione	6.4−2.6[b]
2-methylanthracene-9,10-dione	5.9−2.3[b]
benzanthrone or isomer	22.9−11.1[b]
methylbenzanthrone or isomer	2.6−2.0[b]
benz[*a*]anthracene-7,12-dione	1.7−0.6[b]
phenanthrene aldehyde or isomer	2.4[c]
Aromatic Semipolar Subfraction 5a	
anthracene-9,10-dione	25.5−10.3[b]
2-methylanthracene-9,10-dione	7.9−3.1[b]
7*H*-benz[*de*]anthracen-7-one	2.4−1.5[b]
11*H*-benzo[*b*]fluoren-11-one	57.8−33.5[b]
methylbenanthrone or isomer	0.8−0.4[b]
cyclopenta[*ghi*]perylenone or isomers	6.7[c]
phenanthrene aldehyde or isomer	5.0[c]
pyrene aldehyde or isomers	10.0[c]
Aromatic Semipolar Subfraction 8a	
phenalenone	182−93.0[b]
7*H*-benz[*de*]anthracen-7-one	0.8−0.5[b]
11*H*-benzo[*a*]fluoren-11-one	1.5−0.8[b]
11*H*-benzo[*b*]fluoren-11-one	0.5−0.3[b]
benzanthrone or isomers	22.2−19.4
6*H*-benzo[*cd*]pyren-6-one	2.2−1.1[b]
3,4,4a,5-naphthocoumarin or isomer	0.4−0.3[b]
1,8-naphthalic anhydride	99.4[c]
1,10-phenanthrene dicarboxylic acid anhydride or isomer	32.3[c]
Aromatic Semipolar Subfraction 9a	
flourenone	8.4−1.8[b]
phenalenone	1.6−0.8[b]
7*H*-benz[*de*]anthracen-7-one	1.8−1.1[b]
11*H*-benzo[*b*]fluoren-11-one	0.9−0.5[b]
1,8-naphthalic anhydride	1.5[c]
1,10-phenanthrene dicarboxylic acid anhydride or isomer	0.2[c]
Aromatic Semipolar Subfraction 10a	
1,8-naphthalic anhydride	0.4[c]

Targeted Compounds Not Found in Any Semipolar Subfraction

nitronaphthalene isomers	dinitrofluorene isomers
nitrofluorene isomers	dinitropyrene isomers
other nitropyrene isomers	dinitrobenzo[*a*]pyrene isomers
methyl nitropyrene isomers	hydroxynitropyrene isomers
nitrochrysene isomers	nitrofluorenone isomers
nitrobenzo[*a*]pyrene isomers	thioxanthone
	dibenzothiophene isomers

[a] Range of concentrations shown reflect the range of concentrations measured for each compound when quantified in the whole sample extract and in the various subfractionated extracts. Generally, the highest values are those seen in the whole extract as it has been subjected to the least opportunity for losses during separations and processing. Compound concentrations are stated relative to the quantity obtained when processing 1 mg of organic carbon (EOC) contained within the whole ambient composite filter samples prior to extraction. The Los Angeles area 1993 ambient annual average composite airborne fine particle organic carbon concentration was 8.89 μg of EOC/m^3 of air. [b] Compound also measured in another subfraction, so the concentration reported as quantified from the whole sample extract was determined by multiplying the total concentration quantified in the whole extract by the percentage of the compound concentration occurring in the specific subfraction. [c] Compound measurable only in subfractions; not measured in whole sample.

($\times 10^6$)/mg of EOC. One compound identified in this subfraction has been found to be mutagenic at significantly low enough doses to be a contributing mutagen: 6*H*-benzo[*cd*]pyren-6-one contributes 1.63−0.80 IMF ($\times 10^6$)/mg of EOC or 10−5% of the best estimate of the mutagenic potency of this subfraction.

Subfraction 7a also contains mostly oxygenated aromatic compounds. From the targeted list of semipolar compounds sought in the SIM mode, four compounds were identified: 7*H*-benz[*de*]anthracen-7-one, cyclopenta[*cd*]pyren-3(4*H*)-one, 6*H*-benzo[*cd*]pyren-6-one, and tentatively a hydroxynitrofluorenone isomer. In the full-scan mode analysis, 24 additional peaks are visible of which six can be tentatively identified as 3,4-naphthocoumarin or isomer, cyclopenta[*cd*]pyrenedione or isomer, two methylphthalide isomers, and a dimethylphthalide isomer. The mutagenic potency of this subfraction is 14 ± 4 IMF ($\times 10^6$)/mg of EOC, and again 6*H*-benzo[*cd*]pyren-6-one was found to contribute 0.72−0.35 IMF ($\times 10^6$)/mg of EOC or 5−2.5% to the mutagenic potency of this subfraction.

Subfractions 11a and 12a contain only two compounds on the list of targeted semipolar compounds, 1,8-naphthalic anhydride and a nitrobenzocoumarin isomer. Neither of these two compounds contribute significantly to the mutagenic potency of these subfractions, which is 9 ± 4 IMF ($\times 10^6$)/mg of EOC for subfraction 11a and 5 ± 2 IMF ($\times 10^6$)/mg of EOC for subfraction 12a. Other compounds identified in these subfractions using full-scan mode analysis include 1,8-naphthalic amide, 2,3-naphthalic amide, a phthalamide isomer, an azanaphthalenedione isomer, dimethylazanaphthalene isomers, a nitroindole isomer, carvone, hydroxycarvone, hexadecanamide, heptadecanamide, octadecanamide, and (*Z*)-9-octadecenamide. Those compounds for which response factors could be estimated were entered in Table 1.

Aliphatic Semipolar Subfraction. As explained previously, the separation of aliphatic compounds from aromatic compounds by the size exclusion column is not 100% effective, and thus a few polycyclic aromatic compounds (PAC) can be expected to be found in the "aliphatic" fractions. Three targeted PACs identified in this subfraction (3b−12b) are shown in Table 1. There were 35 peaks visible in the full-scan mode chromatogram, and of these peaks the following compounds have been tentatively identified: nonanal, decanal, dodecanal, hexyl butanoate, dioctyl hexadecane dioate, tributyl phosphate, hexadecanol, octadecanol, dodecanol, (3β,24*S*)-stigmast-5-en-3β-ol, and 5α-stigmast-3-one. Very few of these compounds have been tested in the h1A1v2 assay system, and thus an insignificant portion of the subfraction's activity (10 ± 3 IMF ($\times 10^6$)/mg of EOC) can be attributable to specific compounds identified to date. Since there is some overlap of a few aromatic compounds into this fraction, further identification and pure compound bioassay testing of these compounds may help to explain the source of this subfraction's activity.

Aromatic Polar Subfraction. The organic compounds identified in the mutagenic aromatic polar subfraction (13a) are shown in Table 1 and include naphthalic anhydride isomers, a 202 μ PAC anhydride isomer, naphthalic acid isomers, vanillin, as well as aldehydes such as nonanal and decanal. The cyclic compounds caffeine and cocaine are found in this subfraction, indicating their presence at measurable levels in Los Angeles outdoor air. In addition to those compounds quantified in Table 1, another 25 peaks were observed in the full-scan chromatogram, and those tentatively identified include C_{12}−C_{18} alkanoic acids, 2-(2-butoxyethoxy)ethanol, caprolactam, (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol, and triphenyl phosphine oxide. Again, no significant amount of this subfraction's activity (5 ± 2 IMF ($\times 10^6$)/mg of EOC) can be assigned to identified compounds; however, only two of the compounds identified have been tested in the h1A1v2 human cell mutation assay to date.

Aliphatic Polar Subfraction. The organic compounds identified in the aliphatic polar subfraction are shown in Table 1 and include nonanal, decanal, and naphthalic anhydride isomers. Nicotine, caffeine, and cocaine also are detected in this fraction. In addition to those compounds quantified in Table 1, another 25 peaks were observed in the full-scan chromatogram, and those tentatively identified include phthalamide, 2-(2-butoxyethoxy)ethanol, 2-[2-(2-methoxyethoxy)ethoxy]ethanol, 2-[2-(2-ethoxyethoxy)ethoxy]-ethanol, 2-[2-(2-butoxyethoxy)ethoxy]ethanol, nonanamide, palmitamide, oleamide, glyceryl monopalmitate, glyceryl monostearate, and 2-butoxyethanol phosphate (3:1). The mutagenic potency of this subfraction was 13 ± 3 IMF ($\times 10^6$)/mg of EOC, and none of this activity can be attributed to specific compounds found within this subfraction as there has been very limited testing of polar compounds in the h1A1v2 assay to date. The size exclusion column produces a less well-defined separation between aromatic and aliphatic compounds in this polarity range, as many of the same compounds appear to some extent in both fractions. Therefore, the observation that there is significantly more mutagenic potency in the aliphatic polar subfraction than there is in the aromatic polar subfractions does not necessarily mean that the polar mutagens are aliphatic in nature.

Discussion

The aromatic nonpolar 1 and nonpolar 2 subfractions that contain the unsubstituted PAC contribute a large portion of the mutagenicity of the whole atmospheric aerosol sample. This is consistent with previous results reported for assays involving mammalian cells and whole mammalian animals (*9*, *10*, *20*, *25*−*27*). Six specific PAC that have been tested as pure compounds in the h1A1v2 human cell assay are among the most important mutagens identified to date in the atmospheric sample. Under the assumption of additive effects, these six compounds together could account for an induced mutant fraction per unit of organic aerosol supplied to the h1A1v2 assay of about 24−7.6 IMF ($\times 10^6$)/mg of EOC. That can be compared to the whole sample mutagenic potency of 150 IMF ($\times 10^6$)/mg of EOC or to the summation of the potencies of the fractionated samples, which is in the range of 231−243 IMF ($\times 10^6$)/mg of EOC. Among these PAC, cyclopenta[*cd*]pyrene (12.9−5.31 IMF ($\times 10^6$)/mg of EOC) is the most important contributor to the mutagenic potency of the sample, followed by benzo[*a*]pyrene (3.72−0.87 IMF ($\times 10^6$)/mg of EOC), benzo[*ghi*]perylene (2.43−0.36 IMF ($\times 10^6$)/mg of EOC), benzo[*b*]fluoranthene (2.06−0.51 IMF ($\times 10^6$)/mg of EOC), indeno[1,2,3-*cd*]pyrene (1.73−0.23 IMF ($\times 10^6$)/mg of EOC), and benzo[*k*]fluoranthene (1.17−0.26 IMF ($\times 10^6$)/mg of EOC). Cyclopenta[*cd*]pyrene, benzo[*a*]-pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene historically have been emitted largely from noncatalyst-equipped gasoline-powered motor vehicles in Los Angeles, while emissions of benzo[*b*]fluoranthene and benzo[*k*]-fluoranthene have been dominated by vehicle exhaust plus natural gas combustion aerosol (*39*). Many other unsubstituted PAC and substituted PAC that have yet to be tested in the h1A1v2 human cell assay are present in the atmospheric samples. If these compounds are further characterized by testing in the human cell assay, there is the prospect that much more of the total mutagenic activity of the Los Angeles area atmospheric aerosol can be explained. This is particularly true since the substituted PAC tested to date are generally more potent mutagens than their unsubstituted relatives, and many of the substituted PAC remain to be tested in the human cell assay.

Important mutagens also are found in the fractions beyond those containing mainly unsubstituted PAC. The

four-way separation of the whole sample shows that more than half of the mutagenicity may reside within the semipolar and polar compound groups. Further subfractionation of the semipolar and polar fractions shows that these mutagens are distributed widely across 8 of the 16 semipolar and polar subfractions studied. The identity of the semipolar and polar mutagenic compounds is particularly hard to establish. Few pure compound standards are available for semipolar and polar aromatics, yet such compounds are needed for both positive chemical identification and pure compound testing in the h1A1v2 assay. Nevertheless, a few important semipolar mutagens have been identified and quantified in the atmospheric samples, including 2-nitrofluoranthene (1.27−0.12 IMF ($\times 10^6$)/mg of EOC) and 6*H*-benzo[*cd*]pyren-6-one (summed potency over subfractions 6a and 7a of 2.35−1.15 IMF ($\times 10^6$)/mg of EOC). 2-Nitrofluoranthene is a product of atmospheric chemical reactions in Los Angeles (*30*), while 6*H*-benzo[*cd*]pyren-6-one historically has been emitted largely by noncatalyst gasoline-powered motor vehicles in Los Angeles (*39*).

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