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Polar Organic Matter in Airborne Particles: Chemical Characterization and Mutagenic Activity

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CHEMICAL CHARACTERIZATION AND MUTAGENIC ACTIVITY

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ABSTRACT

Polar organic matter (extracted from inhalable particles collected in Elizabeth NJ and National Institute of Standards and Technology Standard Reference Material SRM 1649) has been characterized by determining elemental and ionic composition, chemical classes and mutagenic activity. The acetone extracts of SRM1649 and Elizabeth NJ samples were 46 and 40% carbon, respectively. When compared to the NJ extract, the SRM extract was enriched in aldehydes and ketones and deficient in carboxylic acids. Significant amounts of organic nitrogen were found in both extracts. Infrared spectra and class tests suggested the presence of nitro compounds, organic nitrates or nitrites, amines, and amides. Fluorescence suggested the presence of aromatic species. For SRM 1649 the acetone extracts accounted for 36% (-S9) and 40% (+S9) of the mutagenic activity in the Ames assay with TA98 (specific mutagenic activity) The acetone extract of SRM 1649 had about four times greater than that from the NJ particles. Both extracts showed substantial decreases in mutagenic activity when tested with nitroreductase-deficient strains of TA98. A simple resolubilization of the NJ extract concentrated the biologically active components into the least polar of three fractions.

INTRODUCTION

Polar organic matter, soluble only in acetone, has been found to account for 30 to 60% of the organic-solvent extractable mass of airborne particles collected in cities in New Jersey and in New York City[1,2] and 30 to 50% of the direct-acting mutagenic activity in the Ames bioassay with TA 98[3,4]. More significantly, this material has been shown to transform mammalian cells *in vitro*.[5,6] Although there is reason to suspect that particulate polar organic matter may be of significance to human health, little is known about its chemical composition.

Particulate polar organic matter has been characterized to a limited extent with respect to oxidized organic compounds by several investigators. Wauters *et al.*[7] extracted airborne particles first with dichloromethane and then with methanol. The methanol-soluble (polar) material was methylated and then analyzed by gas chromatography-mass spectrometry (GC/MS) using both electron impact and chemical ionization modes. Aliphatic and aromatic mono- and di-carboxylic acids and a number of polyols were identified, as well as hydroxy derivatives of mono and dicarboxylic acids. Yokouchi and Ambe[8] used the same extraction procedures as Wauters *et al.*,[7] but redissolved the methanol extract in dichloromethane. The resolublized extract was analyzed by GC and GC/MS after reaction with diazomethane to esterify carboxylic acids. Dicarboxylic acids accounted for 11% of the methanol extract mass, but some hydroxy and keto derivatives of aromatic monocarboxylic acids were also found, in addition to furoic and tetrahydrofuroic acids.

Although many carboxylic acids were identified by these two investigations, a complete mass balance, in terms of organic and inorganic components, has not been achieved, nor do we have information on how much carbon is present and must be accounted for in these extracts. Furthermore, it would be useful to have more information on the chemical classes that are present in the polar extracts so that more complete organic analyses might be done, i.e., by selecting appropriate derivatizing agents for classes which have not been investigated to date. Information on elemental and inorganic composition is also needed for a more complete characterization and mass balance.

The objectives of the work reported here were to characterize polar organic matter from two types of urban airborne particles with respect to elemental composition, organic chemical and polarity classes, and inorganic ions. Two types of particulate matter were used in this investigation: filter samples of inhalable particulate matter collected in Elizabeth NJ[9] during the winter of 1983 and National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1649, Washington Urban Air Particulate Material. The particulate matter was first extracted with non-polar solvent(s) and then with acetone to extract the polar organic matter. Since the ultimate goal of this work is to identify specific mutagenic compounds, the mutagenic activity of the extracts was also determined using the Salmonella microsuspension assay,[10-12] using tester strains TA 98, TA 98 NR and TA98 1,8-DNP6. The latter two are nitroreductase deficient strains which can be used to indicate the presence of mutagens with nitro or dinitro groups.[13,14]

EXPERIMENTAL MATERIALS AND METHODS

Sources of particles, sampling, extraction, storage of extracts. The Elizabeth NJ samples were collected on pre-cleaned glass fiber filters for 24-hour periods over *six* weeks, using a hi-volume air sampler with a size-selective inlet (D_{50} = 15 μm).[9] Individual filters were Soxhlet-extracted sequentially with cyclohexane, dichloromethane, and acetone.[1,2] Each extract was filtered and reduced in volume to 10.0 ml using a rotary evaporator. Extract masses were determined with a Cahn microbalance, Model 25, by weighing the residue of duplicate 100 μl aliquots of the extracts taken to dryness on a slide warmer. Filters and extracts were stored in a freezer at -30°C, in the dark. For this research, a composite sample of the acetone-soluble extracts from Elizabeth NJ was prepared based on equal air volumes for each of the 39 days of the sampling period. The total composite mass was 479 mg, corresponding to sampling 44,400 m^3 air. The SRM 1649 acetone extract was prepared by sequential Soxhlet extraction of one gram portions of particles with dichloromethane followed by acetone, filtration and rotary evaporation of excess solvent. Extract samples were stored in the freezer. Previous work had shown that the mass of material extracted with dichloromethane alone is equivalent to the sum of the masses extracted with cyclohexane followed by dichloromethane.[15]

Fractionation by solvent polarity. The Elizabeth NJ acetone extract was subjected to a simple fractionation by re-solubilization in two organic solvents of different polarity for exploratory purposes. This is shown schematically in Figure 1. An aliquot of the composite extract (104.5 mg) was taken to dryness using a rotary evaporator. The residue was then sonicated with 3 ml of dichloromethane and the dichloromethane-soluble fraction, designated P (polar fraction), was removed. Six milliliters of acetone was then added to the insoluble residue left behind in the flask (R1), and the residue was sonicated. The material soluble in that volume of acetone was designated the VP (very polar) fraction. The residue left behind after treatment with the two solvents was designated the R2 (residual) fraction. The R2 fraction was not characterized further. The mass of each solubilized fraction was determined for duplicate 100 microliter aliquots.

Elemental analyses. Portions of the composite acetone extracts (10 to 15 mg) from Elizabeth NJ and from SRM 1649 were analyzed for C, H and N by Schwarzkopf Microanalytical Laboratory, Woodside, NY. An aliquot of Elizabeth fraction P and an acetone filter-solvent blank (from sequential extraction) were analyzed. VP was lost in shipping.

H^+ determinations. Aliquots of the SRM 1649 extract and the Elizabeth extract and its P and VP fractions were taken to dryness in a warm water bath. Hydrogen ion concentration was determined in a series of sonicated solutions, ranging from 0.25 to 1 mg ml^{-1} of extract per milliliter of de-ionized water. A Cornell X-El electrode was calibrated using standard buffer solutions before each determination. Reported results are the averages based on moles per gram extract.

Ion chromatography. Aliquots of the acetone extracts and fractions were dried in air and dissolved in deionized water by sonication to give concentrations of 0.02 to 0.14 mg/ml. Ammonium, sodium, potassium, nitrate, chloride and sulfate ions were determined by ion chromatography on a Dionex Autoion System 12 Analyzer with AS-3 and SC-3 columns with

micromembrane suppressors. The cation eluant was 5 mM HCl. The anion eluant was 3 mM HCO_3^- and 2.4 mM $CO_3^=$.

High performance liquid chromatography. Standard compounds, whole extracts and fractions were analyzed on a Hewlett-Packard Model 1090M high pressure liquid chromatograph equipped with a DR 5 solvent delivery system, a diode array ultraviolet/visible detector, a fluorescence detector (Model 1046A) and Model 79994A LC Workstation software. Samples were chromatographed on a Vydac 201TP52 C_{18} reversed-phase microbore column, 2.1 mm I.D. x 15 cm and 5 micron diameter particles, using gradient elution. The analytical column was preceded by a guard cartridge column filled with 10 micron diameter Vydac 201TP packing material (purchased from Alltech Associates). The initial solvent composition was 5% acetonitrile in water. From 3 to 13 min the solvent composition was changed linearly to 100% acetonitrile and held there for 5 min. Gradient reversal and column equilibration were complete 25 min after injection. Flow through the column was 0.5 ml/min. Three absorbance wavelengths were used for detection: 205 nm, 230 nm, and 254 nm. The fluorescence detector used an excitation wavelength of 250 nm, and all emitted light above 305 nm was collected. Retention times were determined for a number of polar aromatic standard compounds at concentrations of about 0.4 mg ml^{-1} each. These compounds provide reference points for the polarity of the extracts when characterized by HPLC.

Fourier-transform infrared spectra. FTIR spectra of the whole extracts and of fractions were recorded on a Mattson Cygnus 25 spectrometer using 300-700 μg of extract (dry weight) mixed with about 50 mg dry ground KBr. Pellets were prepared with a mini-press with a window diameter of 8 mm.

Chemical class tests. Organic chemical colorimetric class tests (spot tests), as described by Feigl,[16] were also used to provide information on the organic classes present in the acetone extracts and fractions. The class tests were done in triplicate on 100 microliter aliquots (210 micrograms)

of the unfractionated Elizabeth acetone extract, alone and while mixed with 100 microliter standard solutions at each concentration. The three standard solutions were analyzed at the same time, for a total of nine spots per class. Spot tests were carried out in a glazed white porcelain spot test plate or in 8 ml glass vials. Extracts of blank filters were also analyzed. Sensitivity was estimated from results for aliquots of standard compounds at 1, 10 and 100 microgram per test. For the SRM 1649 acetone extract, amounts used for each test varied from 50 to 180 micrograms, as indicated below. Not all tests were performed on the SRM extract.

Bioassays. The mutagenic activity of the extracts was determined using a *Salmonella* microsuspension assay previously described.[10-12] The procedure is at least ten times more sensitive than the standard plate incorporation test based on absolute amounts of material added per plate. In addition to tester strain TA 98, the nitroreductase-deficient tester strains TA98 NR and TA 98 1,8-DNP_6 were used. (The nitroreductase-deficient strains had been kindly provided by H. Rosenkranz, Cleveland OH.) These strains lack endogenous enzymes responsible for activating certain nitro-substituted compounds.[13,14]

Aliquots of the acetone extracts were first dried under a stream of nitrogen and then 20 to 50 μl of dimethylsulfoxide (DMSO) was added, depending upon the desired concentration. The DMSO solutions were serially diluted in DMSO for the other doses. For the microsuspension assay, 0.1 ml of S9 solution or buffer (PBS., 0.15M, pH 7.4), 0.1 ml of concentrated bacteria, and 0.005 ml of the filter extract were added to a 12 x 75 mm glass culture tube. The mixture was incubated for 90 minutes at 37°C, and molten top agar was added to each tube. The top agar-incubation mix solution was poured onto a standard Vogel Bonner bottom agar, as described by Ames, *et al.*[17] The bacterial tester strains used were routinely characterized for strain markers. The S9 (40.5 mg protein/ml) was prepared from the Aroclor-induced livers of male Sprague-Dawley rats and was used throughout. 300μg of protein was used per ml of S9 mix. Specific mass mutagenic activity was calculated by linear regression analysis of the linear portion of the dose-response curves.

Size exclusion chromatography. Size-exclusion chromatography was used to characterize the polar extracts with respect to molecular weight distribution. An ultrastyragel 100 A column (30 cm in length and 7.8 mm in internal diameter) with a differential refractive index detector was used. The solvent was tetrahydrofuran at 1.0 ml min^{-1}.[18]

RESULTS

The acetone-soluble matter from SRM 1649 comprised 33% of the total organic soluble matter and 2.8% of the particle mass. Previous work on the Elizabeth NJ samples had shown that the acetone soluble matter averaged 55% of the total organic-soluble matter (cyclohexane, dichloromethane and acetone, in sequence) from the collected particulate matter and about 20% of the particle mass.[2] Five and three percent, respectively, of the mass of the acetone-solubles of the SRM 1649 and Elizabeth particles were characterized as high molecular weight (>800 amu) material when analyzed by size-exclusion chromatography.

Table I shows the ionic composition of the two acetone extracts (Elizabeth and SRM 1649). Both extracts were fairly acidic. The Elizabeth extract was more acidic, with a pH of about 3.7 at 0.25 mg extract per ml water; the extract of SRM particles had a pH of 4.4 at 0.25 mg extract per ml water. The measured ions constituted 7 and 18% of the acetone extractable masses of the SRM 1649 and Elizabeth and samples, respectively. Nitrate ions accounted for more than half of the inorganic mass for both samples. Daisey *et al.*[19] have previously reported similar results for acetone-soluble particulate organic matter from a limited number of samples collected in New York and New Jersey.

Substantial differences in ionic composition were observed for the fractions obtained by re-solubilization of the Elizabeth acetone extract. The very polar (VP) fraction had three times the ionic mass of the polar (P) fraction (Table I). However, half of the total ionic mass remained in the unanalyzed residue (R2). P and VP were equally acidic. About half of the acetone extract mass was recovered in P and one-quarter was recovered in VP.

Results of the C, H, and N analyses are also presented in Table I. Empirical formulas for the organic components of the extracts were calculated in order to compare the composition of these polar extracts to the empirical formula reported by Sawicki,[20] for a composite of non-polar benzene-soluble extracts from total suspended particulate matter samples collected in 200 U.S. cities ($C_{32}H_{47}N_{0.16}O_{3.3}$). The calculated formulas have been normalized to give an integer value of 32 for carbon. The percentage of hydrogen in ammonium ion has been subtracted from the total percent hydrogen, and the percentage of ionic nitrogen was subtracted from the total percentage of nitrogen. The difference between the sum of (organic C, H, N, ionic mass) and 100 percent was assumed to be oxygen. Although the assumption may lead to an overestimate of oxygen content, the FTIR spectra also indicated high proportions of oxygen in the extracts.

When characterized by HPLC with ultraviolet detection, the acetone-soluble extract of the SRM 1649 particles showed a doublet absorbance peak starting at the retention time corresponding to the void volume of the column and tailing in a manner consistent with the presence of ionized species (Figure 2). This material eluted at retention times close to that for 5-nitrovanillin (1.6 min). For all peaks, the intensity decreased in order 205>230>254 nm. The Elizabeth NJ unfractionated extract showed two major absorbance peaks at widely-separated retention times. The P fraction of the Elizabeth extract displayed only the later-eluting peak, eluting near the reference compounds benzoquinoline and acridine, but the major feature for the VP fraction eluted at the column void volume. The chromatograms clearly showed that separation of the ultraviolet chromophore-containing classes of compounds was possible by exploiting the difference in solubility of extract components based on solvent polarity. For all samples, including SRM 1649, fluorescent peaks were also observed at the same retention times as the absorbance peaks, but additional fluorescent peaks were observed between 7 and 10 minutes (not shown). For the Elizabeth NJ extract, the fluorescence between 7 and 10 minutes was more intense than that between 2 and 4 minutes. The fluorescence maximum of P occurred later than the maximum for VP, but they had very similar intensity.

The infrared absorbance spectra of the acetone extracts are shown in Figure 3. The numbers near the main features identify peaks which are listed and assigned in Table II. Functional group assignments are based on the literature.[21,22] Besides the extracts and fractions, a salt mixture with the same approximate ionic composition as the unfractionated Elizabeth extract was analyzed so that the inorganic species could be assigned more easily in the extracts and fractions. Both SRM 1649 and Elizabeth unfractionated acetone extracts showed peaks consistent with the presence of oxygenated organic species such as carboxylic acids, alcohols, aldehydes, ketones and esters; and nitrogen-containing species such as nitro groups, organic nitrates, amides, amines and inorganic ammonium and nitrate salts. No extract or fraction showed strong evidence for anhydrides, peroxides or carbonates. The SRM extract had weaker absorbance per mg than the Elizabeth extract for OH, CH, C=O, and NO_3^-, but about the same value for NH_4^+. This result is consistent with the relative amounts of ammonium and nitrate found by ion chromatography (Table I) and the differences in calculated empirical formulas. The Elizabeth extract had stronger absorbances assignable to carboxylic acids and carboxylate ions than the SRM extract.

The P fraction had enhanced CH and CO structure compared to the VP fraction and the original acetone extract, and P showed evidence for the presence of carboxylic acids, esters, ketones, aldehydes, alkyl groups and some NO_x containing species such as nitro groups and organic nitrates. VP also displayed clear evidence for oxygenated species containing carbonyl and carboxyl groups, in addition to NH groups (ammonium, amines and amides) and NO_3^-. These results are also consistent with the ion chromatography data for the fractions (Table I).

Table III presents the semi-quantitative results for class analyses of the unfractionated acetone extracts and the Elizabeth P and VP fractions. The amounts of each class have been estimated by comparison to the response of the standard compound at 3 or 4 concentrations. The SRM 1649 extract contained a higher proportion of aldehydes and ketones than the Elizabeth extract which had enhanced carboxylic acid and phenol content when compared to SRM 1649. These differences are consistent with the FTIR spectra. There was evidence for the presence of alcohols in the

Elizabeth extract; the SRM 1649 extract was not tested for this class due to the large mass required for the test and the limited amount of extract that had been prepared. Neither extract had detectable quantities of esters. The Elizabeth extract was tested for anhydrides, quinones and amines; no detectable amounts were found. Not listed in Table III are positive results for sulfones and amides in the unfractionated Elizabeth extract. The positive amide test, in which amide is hydrolyzed to and detected as ammonium ion, was considered inconclusive because ammonium was found by ion-chromatography (Table I).

Table IV presents the specific mutagenic activity for *Salmonella* strain TA 98 in the microsuspension assay, with and without added S9, for the SRM 1649 dichloromethane (DCM) and acetone extracts, and for the Elizabeth acetone extract and its fractions. The DCM extract from SRM 1649 was tested along with the acetone so that comparisons could be made to the work of others who have extracted SRM 1649 with DCM followed by a polar organic solvent. For the SRM 1649, the specific mutagenic activities of the DCM and acetone were similar with TA98 (-S9) addition of S9 reduced the activity of the DCM-solubles by about a third. The SRM acetone extract was about 3- to 4-times more potent as a mutagen than the Elizabeth NJ acetone extract. Addition of S9 mix reduced the mutagenic activity of the Elizabeth acetone extract by almost half.

The specific mutagenic activities of both extracts decreased when tested in nitroreductase-deficient strains of TA98 (Table V). For both extracts the activities in TA98 NR decreased to approximately 30 percent of the activities of TA98 (both minus S9). The activities of TA98 1,8-DNP6 were 13 and 9 percent of the activities in TA98 for SRM 1649 and Elizabeth samples, respectively.

DISCUSSION

Chemical composition: For SRM 1649, the non-polar organics, soluble in DCM, accounted for 5.7% of the particle mass, and acetone-solubles accounted for another 2.8% (Table I). These results are consistent with those reported by Zinbo *et al.*[18] for the methanol extract of SRM 1649 (previously extracted with DCM), which averaged 6.1% of the particle mass and 52% of the total

extractable mass. Methanol extracts more of the inorganic components of airborne particulate matter, and therefore the percentage of methanol-solubles was higher than the percentage of acetone-solubles in SRM 1649. In contrast, the non-polar and polar organic-solubles accounted for 17% and 20%, respectively, of the particle mass of the Elizabeth particulate matter.[2] The SRM 1649 particulate matter was collected in filter bags and screened through a fine mesh sieve;[23] thus, it included much larger particles than the Elizabeth samples which were collected with a size-selective inlet with $D_{50} \leq 15$ μm. Since the organic components of airborne particles are concentrated in the smaller size particles, the SRM 1649 particle mass had a smaller proportion of organic soluble material. For both types of particles, however, the acetone-solubles constituted a substantial fraction of the total organic-soluble matter, 33% and 55 %, respectively, for the SRM 1649 and the Elizabeth samples.

Sodium, ammonium, potassium and nitrate were the most abundant ionic species for both SRM 1649 and Elizabeth samples. On the basis of equivalents per gram of extract, there was an anion deficit for the extract of SRM 1649 and a cation deficit for the Elizabeth extract. Carboxylate anions were not determined by ion chromatography, but their presence was suggested by the FTIR spectra and class tests for both extracts. Their presence could also help to explain the anion deficit observed for the extract of SRM particles. Hydrolysis of amides would also lead to excess ammonium ion concentration. The cation deficit found in the Elizabeth extract may have been due to the presence of other cations such as calcium and magnesium which were not measured. Alternatively, or in addition, excess nitrate could have been generated by the hydrolysis of organic nitrate esters when the extracts were dissolved in water for ion chromatographic analysis.

The major components of the acetone extracts, however, were oxygen-containing organic compounds. This conclusion is based on the available elemental composition data, as well as characteristic infrared absorbances and the results of chemical class tests. Carbon accounted for 46% of the polar organics (acetone-solubles) from SRM 1649 and 40% for the Elizabeth extract. In comparison, based on the data reported by Sawicki,[20] non-polar organics in urban particulate

matter contained about 79% carbon. The polar extracts contained much greater proportions of oxygen and nitrogen, than did the non-polar extracts. Based on the estimated molecular formulas shown in Table I, non-ionic nitrogen accounted for about 4% of the mass of the acetone-solubles while oxygen accounted for about 35% on average. There were, however, some differences in the elemental composition of the Elizabeth and SRM extracts, with somewhat higher proportions of non-ionic nitrogen and oxygen found in the SRM 1649 than in the Elizabeth extract.

The FTIR spectra and class tests also indicated that organic compounds accounted for most of the acetone-soluble extract masses and that these compounds were highly oxidized. The large proportions of oxygen in the polar extracts were distributed among carboxylic acid, aldehyde, ketone, alcohol, phenol and possibly ester functional groups. These results are consistent with those of previous investigators who have reported the presence of carboxylic acids in polar extracts of airborne particles.[7,8] However, this study indicates that aldehydes, ketones and phenols are also important oxidized organic components of the polar extracts.

The chemical class analysis results reported here for polar solvent extracts are also consistent with the results reported by Schuetzle *et al.*[24] who used computer-controlled mass spectrometric thermal analysis to characterize aerosol samples less than and greater than 1-2 μm in diameter which had been collected in Los Angeles during a period of photochemical smog. In this method, organic compounds were directly volatilized into a mass spectrometer by heating a gold plate on which the particles were collected. Many bifunctional organic compounds, with acid, aldehyde, nitrite and nitrate functional groups, were identified in the sub-micron aerosol.

The differences between the elemental and ionic analyses indicated the presence of significant amounts of organic nitrogen compounds in the polar extracts. The FTIR spectra and the class tests suggested that these may be organic nitro, nitrate or nitrite compounds, in addition to amines or amides. However, some of their identifiying absorbances were somewhat obscured by strong bands for ammonium and nitrate ions. Schuetzle *et al.*[24] indentified organic nitrate and nitrite

compounds in airborne particles. The fluorescent peaks detected in the HPLC chromatograms suggest the presence of polycyclic aromatic rings; these compounds are likely to be oxidized derivatives of polycyclic aromatic hydrocarbons and/or aza-arenes. Butler and co-workers[25] have previously reported evidence for the presence of aza-arenes in acetone extracts of particulate matter collected in Elizabeth. FTIR bands for aza-arenes would probably not be detected in the complex mixture of the unfractionated extract.

The acetone extracts of SRM 1649 and Elizabeth NJ had some significant differences in chemical composition. For example, the SRM 1649 extract was enriched in aldehydes and ketones, compared to the Elizabeth extract. The Elizabeth extract was enriched in carboxylic acids and carboxylate ions and had stronger evidence for alcohols and phenols. The SRM extract also had a smaller percentage ionic content than the Elizabeth extract.

The very simple resolubilization fractionation of the Elizabeth extract produced three fractions which differed in chemical composition. It should be noted that this fractionation was not optimized for solvent volumes and mass recovery as it was exploratory in nature. Compared to the unfractionated acetone extract, the P fraction was enriched in carbon. P had 92% of the carbon but only 14% of the ionic mass. Half of the inorganic mass remained in the R2 rather than the P or VP fractions. The calculated empirical formula for the Elizabeth P fraction had less non-ionic nitrogen and oxygen than the original acetone extract. Although the VP fraction was lost in shipping and, therefore not analyzed for C, H, and N, the difference between the inorganic nitrogen in the whole extract and the P fraction suggests that much of the non-ionic nitrogen went into the VP and/or R2 fractions.

The P and VP fractions gave responses to the chemical class tests that were similar in intensity to those of the unfractionated extract. However, HPLC and infrared spectroscopy indicated that the P and VP fractions differed substantially in polarity, relative amounts of organic functional groups and inorganic species. Because the P fraction was enhanced in CH and C=O and

efficient in OH compared to VP, it is likely that P contained more aldehydes, and ketones, and fewer carboxylic acids than VP. The resolubilization concentrated the less polar species from the unfractionated acetone extract into the P fraction. The differences in elemental composition and HPLC retention time indicated that substantial amounts of organic oxygen and nitrogen went into the VP and/or R2 fractions. Such organic species were more polar (possibly multi-functional) and more likely to be ionizable than those found in P. The evidence is consistent with the presence of multi-functional carboxylic acids, amides, and organic nitrates in VP.

Mutagenic Activity. The specific mutagenic activities of the SRM DCM and acetone extracts, reported in Table IV, were comparable to each other with TA98 (-S9); with S9 added, the activity of both extracts was reduced by about one-third. Since the DCM-solubles and acetone-solubles account for 67% and 33%, respectively, of the total organic solubles, the acetone-solubles accounted for about 36% (-S9) and 40% (+S9) of the total mutagenic activity found in the soluble organics, i.e., DCM plus acetone. Similar results have been reported for the distribution of mutagenic activity in SRM 1649 using a slightly different extraction procedure. Nielsen[26] Soxhlet-extracted SRM 1649 particles first with DCM, then again with fresh DCM, and then finally with acetone. Using the standard plate Ames assay, Nielsen found that the extracts accounted for 59%, 22% and 20%, respectively, of the total mutagenic activity with TA-98 (-S9) and 75%, 14%, and 11%, respectively, with TA-98 (+S9).

Some investigators have used a sequence of DCM extraction of SRM 1649 followed by a methanol extraction to recover the more polar organic matter.[18,27] With a Soxhlet extraction, Nishioka and co-workers[27] recovered 5.2% of the SRM 1649 particle mass with DCM and an additional 5.8% with the methanol. The methanol-solubles, however, accounted for only 17% (−S9) and 12% (+S9) of the total mutagenic activity of the two organic-soluble extracts (DCM plus methanol). Zinbo, *et al.*[18] have reported similar results; that is, the polar organic matter recovered with methanol accounts for much less mutagenic activity than does that recovered by acetone. The reasons for this difference are not known and probably cannot be determined until we

have more information on the chemical nature of the polar mutagens. Acetone may solubilize more of the polar mutagens than methanol. Artifact formation could occur for both solvents. Another possibility is that the inorganic materials present in the methanol extracts (which contain more inorganic matter than the acetone extracts) have an inhibitory effect on the mutagenic activity of this fraction.

With the microsuspension assay, the Elizabeth NJ acetone extract had a specific mutagenic activity that was only about one-fourth (Table IV) to one-third (Table V) that of the SRM 1649 acetone extract. (The data of Tables IV and V are from two independent determinations.) In previous studies of the Elizabeth NJ acetone extract,[4] using the standard plate assay, this polar extract had specific activities in TA 98 that were about one-fourth those measured with the microsuspension assay and presented in Table IV [1.55 rev/μg (-S9) and 0.67 rev/μg (+S9)]. In that work the Elizabeth NJ acetone extract accounted for approximately half of the total (non-polar plus polar) mutagenic activity of these particles with TA-98 ($\pm$ S9).

The SRM 1649 and Elizabeth acetone extracts showed substantial decreases in mutagenic activity when tested in nitroreductase-deficient tester strains. The results suggest that niro-containing mutagenic compounds are present in each of the acetone extracts. The specific mutagenic activities for both extracts in TA98NR decreased to approximately 30% of the activities of TA98. The activities in TA98 1,8-DNP6 decreased further to about 10% of the activities of TA98 alone, which indicates the presence of dinitro-substituted compounds.

Nitro-organic compounds have been found to account for some of the mutagenicity of the less polar organic extracts, such as DCM. If the mutagenicity of acetone or methanol extracts of airborne particles is due to the presence of nitro compounds, these nitro compounds must also possess other functional groups such as CO, OH and COOH. Since hydroxy-PAH[28] and nitrated lactone derivatives of PAH[29] have been identified as mutagens in DCM extracts of ambient particles, more polar derivatives of nitrated PAH would be expected in acetone or methanol

extracts. Many of the aza-arenes are also mutagenic. These compounds, which are not easily solubilized by non-polar solvents such as benzene or DCM, may account for some of the activity observed in the polar extracts as well as the organic nitrogen. The observation of fluorescent peaks in acetone extracts indicates the presence of aromatic polar species such as oxygenated nitro PAH or azarenes. Future studies should investigate the nature of the organic nitrogen compounds whose presence is suggested here and their possible relationship to the mutagenicity of the polar extracts.

The simple resolubilization fractionation which was applied to the Elizabeth acetone extract was very effective in concentrating the mutagenic activity into a fraction which was depleted in inorganic material and enriched in organic species. Data presented in Table IV show that 80-90% of the recovered mutagenicity was found in P. The resolubilization fractionation can thus provide a simple method to concentrate the mutagenic activity for bioassay-directed fractionation and also provide a fraction which is reduced in ionic content.

Summary: This study reports evidence that acetone extracts of ambient particles are largely organic in nature, with a variety of oxygen-containing functional groups. In addition to the previously identified carboxylic acids, there is evidence for the presence of aldehydes, ketones, phenols and alcohols. The relatively high percentage of organic nitrogen (inferred by the difference between elemental and ionic nitrogen) also distinguishes the polar from the non-polar extracts of airborne particles. Although the FTIR spectra and class tests suggest that these nitrogen-containing compounds may be nitro compounds, nitrates, nitrites, amides and amines, more specific compound identification is needed to adequately characterize these nitrogenous organics. Previous work by Schuetzle *et al.*[24] suggests that many of these oxygen- and nitrogen-containing organic compounds could be bi- or polyfunctional organic compounds. The acetone extracts investigated here accounted for substantial fractions of the total mutagenic activity of the organic-soluble particulate matter. Identification of the mutagens in the polar organic extracts from airborne particles will require development of a suitable fractionation method for these materials and subsequent bioassay-directed fractionation.

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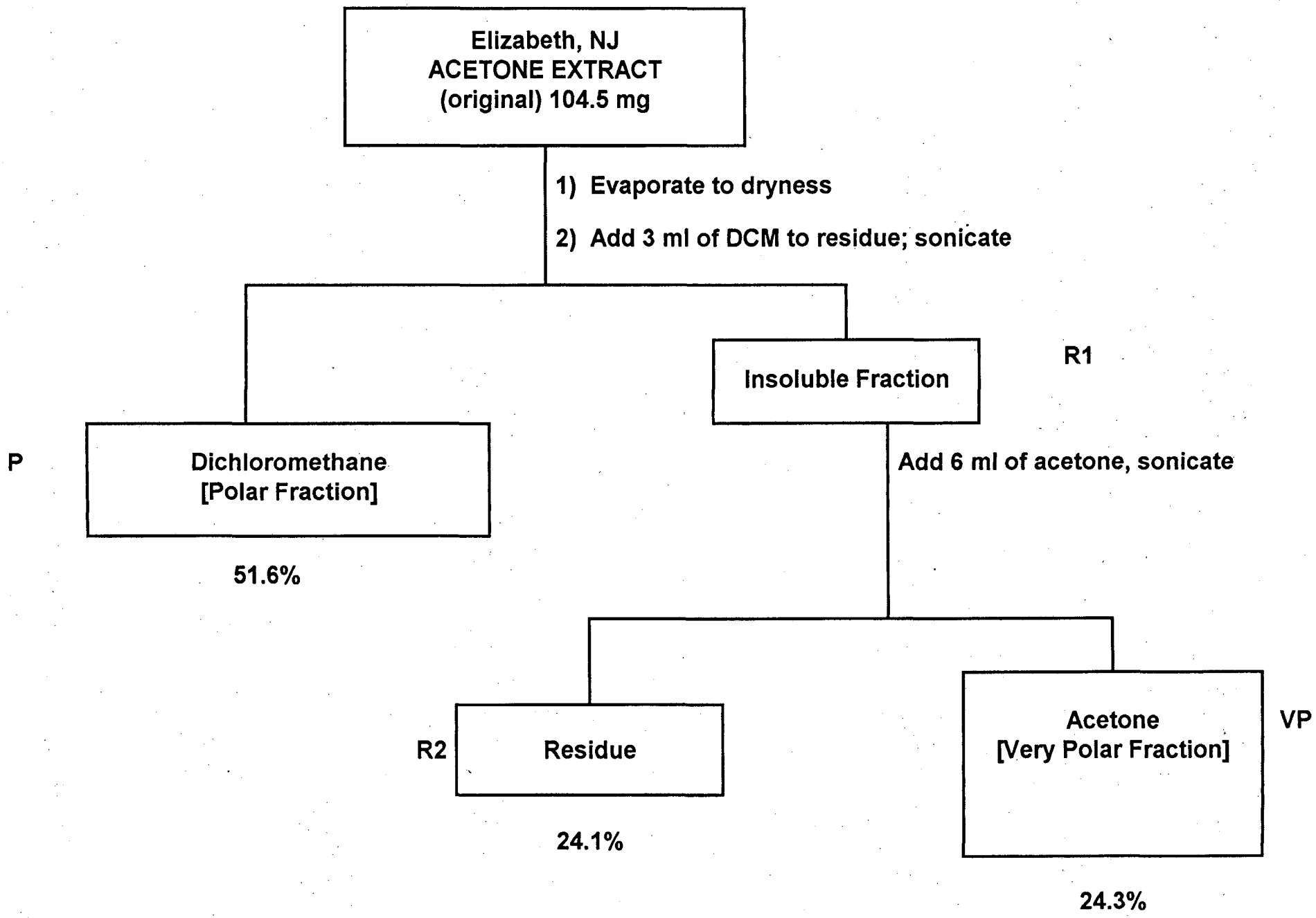

Figure 1. Schematic diagram of the fractionation by solubility.

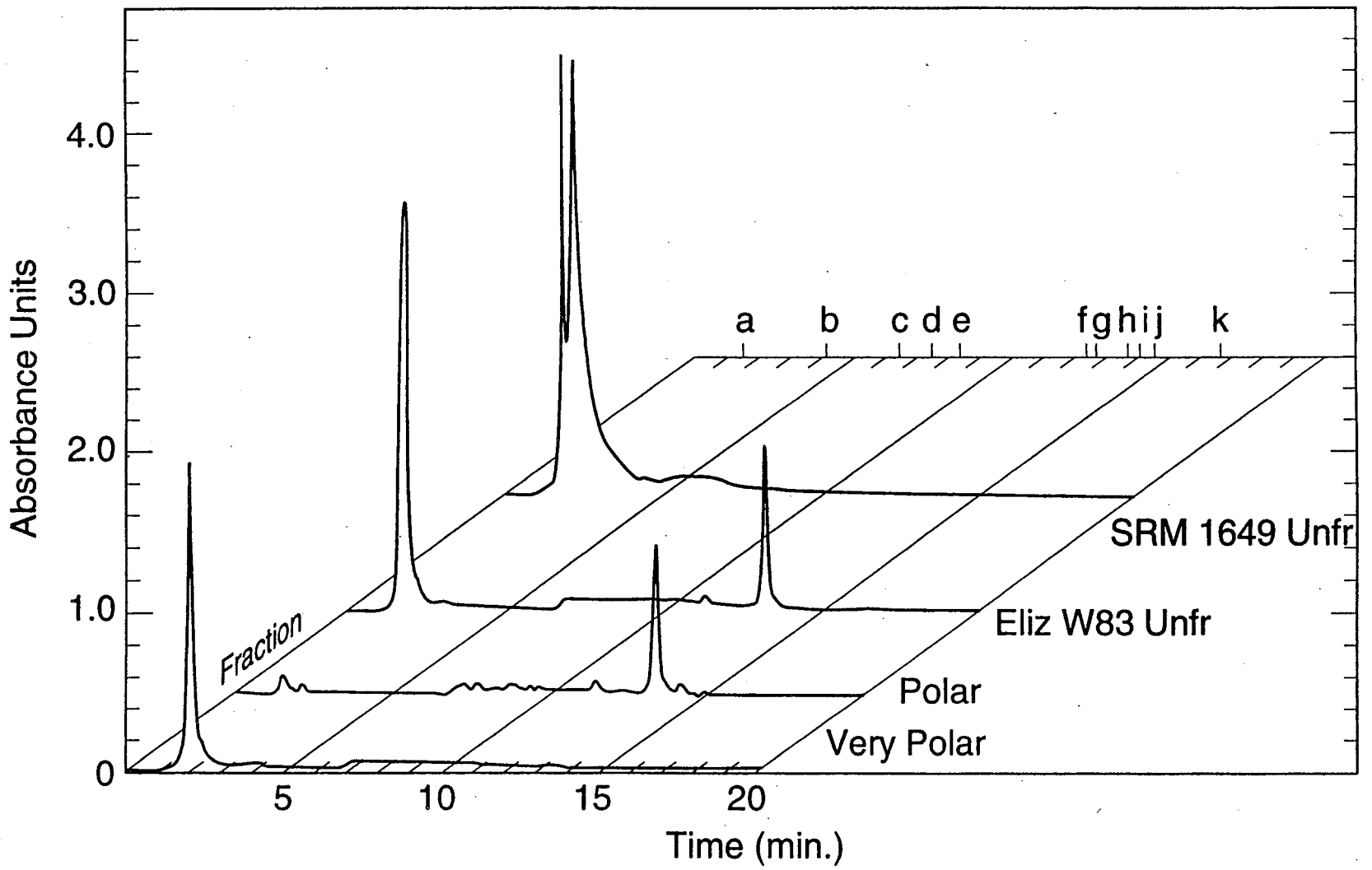

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Figure 2. Reverse-phase high performance liquid chromatographic analysis of acetone extracts of SRM 1649 and Elizabeth NJ particulate matter and its polar and very polar fractions. The ordinate gives absorbance at 205 nm; the abscissa is retention time. The top chromatogram shows the retention times for the aromatic standard compounds: a, 5-nitrovanillin; b, vanillin; c, 2-naphthoic acid; d, 1,4-dihydroxynaphthalene; e, 5-nitroquinoline; f, 2-nitro-6H-dibenzo[b,d]pyran-6-one; g, 2-nitronaphthalene; h, 5,6-benzoquinoline; i, acridine; j, 1-pyrenecarboxaldehyde; and k, dioctylphthalate.

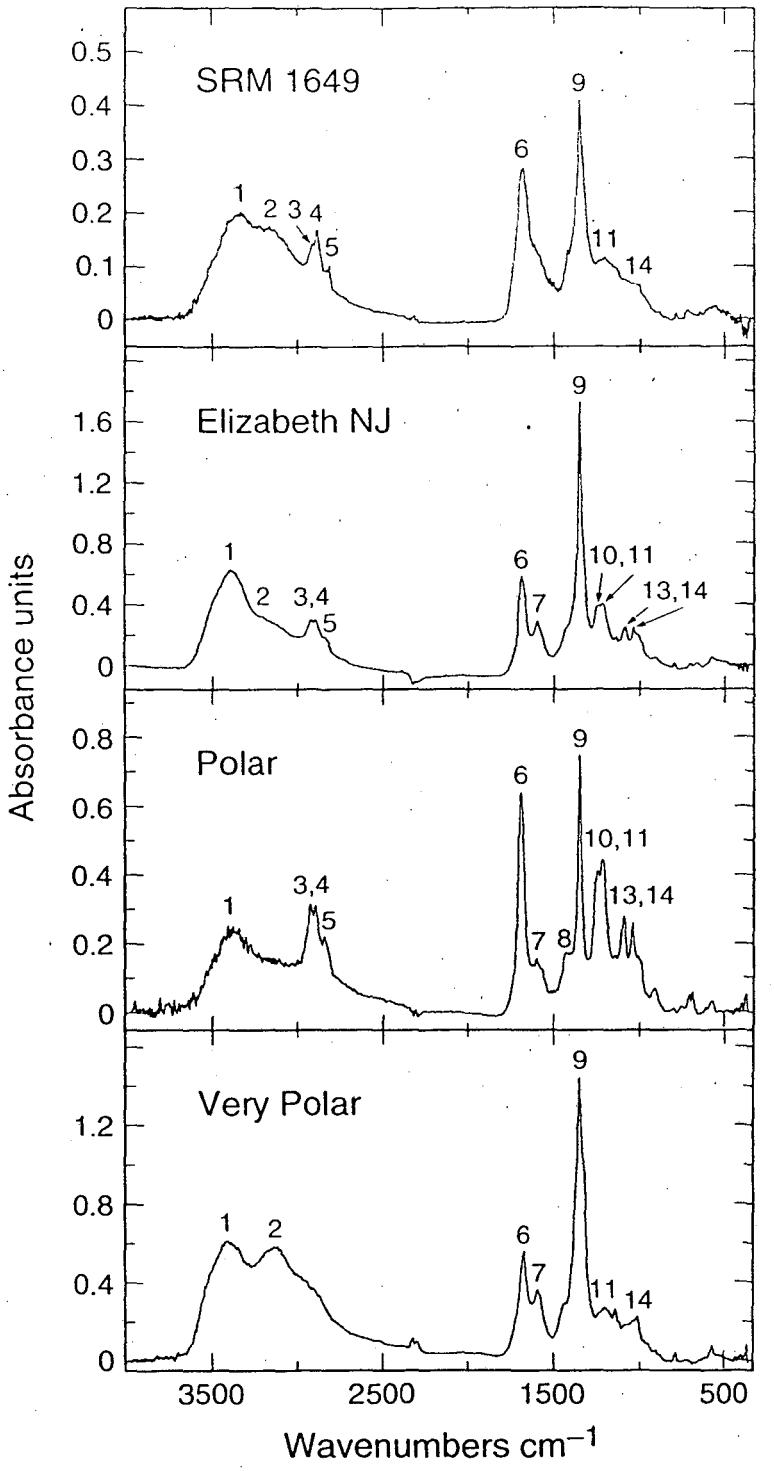

Figure 3. FTIR absorbance spectra for acetone extracts of SRM 1649 and Elizabeth NJ urban particulate matter and its polar and very polar fractions. Numbered features refer to Table II.

Table I. Elemental and Ionic Composition of Acetone-Extracts from SRM 1649 and Airborne Particles.

	Percent of Acetone Extract Mass											Percent of Fraction				
Extract or Fraction	Mass	H^+	NH_4^+	Na^+	K^+	Cl^-	NO_3	SO_4	Sum of Ions	Sum of Cations meq/g	Sum of Anions meq/g	C	H[a]	N[a]	O[a,b]	Molecular formula[b,c]
SRM 1649 Acetone Extract	100	0.02	0.51	0.91	1.05	0.22	4.12	0.10	6.93	1.12	0.75	46.5	4.7 *4.6*	6.5 *5.1*	42.3 *36.9*	$C_{32}H_{38}N_3O_{19}$
Elizabeth NJ Winter, 1983 Acetone Extract	100	0.06	0.58	2.57	0.60	3.58	10.91	0.13	18.4	2.16	2.80	39.9	5.3 *5.2*	6.4 *3.5*	48.4 *33.0*	$C_{32}H_{50}N_{2.4}O_{20}$
P (Polar)	51.6	0.06	0.081	0.015	0.02	0.71	1.59	0.023	2.5	0.66	0.46	74.5	8.7 *8.7*	3.8 *2.7*	13.0 *9.4*	$C_{32}H_{45}NO_3$
VP (Very Polar)	24.3	0.06	0.57	0.30	0.20	2.90	3.00	0.07	7.1	1.09	1.32	*d*	*d*	*d*	*d*	*d*

a. Values in italics are the non-ionic elemental percentages.
b. By difference.
c. For non-ionic components; benzene-soluble organics for composite sample of particulate matter from 200 cities reported as $C_{32}H_{47.4}N_{0.16}O_{3.3}$ by Sawicki, (ref. 20).
d. No data; sample lost in shipping.

Table II. Infrared Qualitative Analysis of SRM 1649 and Elizabeth NJ Winter 1983 and Acetone Extracts.[a]

Code [b]	cm^{-1}	SRM	Elizabeth, NJ	P	VP	Assignment[c]	Possible Species
1	3435	s	s	ms	m	OH, NH	Carboxylic acids, phenols
2	3200	m	w	*d*	m	NH	NH_4^+, amides
3 4 5	2960 2935 2870	} } m }	} } m }	ms	vw	CH Stretch	Aliphatic groups
6	1720	s	s	s	m	C=O, C=N	Carboxylic acids, aldehydes, Ketones, esters, amides
7	1635	w	m	mw	m	Asym Stretch	Ionized acids RCO_2^- Organic nitrates $RONO_2$, (water)
8	1450	w	vw	w	w	Sym Stretch	Ionized acids RCO_2^-, NH_4^+
9 10	1380 1275	s	s }	s	s	N-O Stretch C-O Stretch	$-NO_3^-$, $-NO_3$, $-NO_2$ Carboxylic. acids, esters
11	1285	w	} m }	s	w	NO_2 Sym Stretch CN Stretch OH Def	Organic nitrates Aromatic amines Alcohols
13	1130	*d*	mw	m	*d*	CO Stretch OH Def	Alcohols
14 15	1070 1050	vw	} } mw }	m	w	CO Stretch OH Def	Alcohols

a. s = strong; ms = medium - strong; m = medium; mw = medium - weak; w = weak, vw = very weak
b. Peaks marked on Figure 3
c. Reference 21 and 22
d. Absent

Table III. Chemical Class Test Results for SRM 1649 and Elizabeth NJ Winter 1983 Acetone Extracts. The notation +++ indicates that positive responses exceeded the color change seen with standard compounds at the highest concentrations. The notation + indicates positive responses within the color change range seen for standard compounds.

Class	SRM 1649	Elizabeth NJ[a]				Standard	Detection Limit[b] Micrograms
	μg	μg		P	VP		
Carboxylic acids[c]	≥10[d]	≥100	+++	+++	+++	adipic acid	5
Aldehydes	≥100[e]	≤10	+	+	+	9-anthraldehyde	0.3
Ketones	≤10[e]	≤1	+	+	+	acetophenone	<0.3
Alcohols	nt[f]	≥800[g]	+	+	+	methanol benzylalcohol 2-propanol	80-800[h]
Phenols	≤10[i]	10-100	+	+	+	hydroquinone	7
Anhydrides	nt	≤10	-	-	-	succinic anhydride	10[j]
Esters	<10[j]	≤10	-	-	-	diethylacetamide-malonate	10
Quinones	nt	≤10	-	-	-	p-benzoquinone	10[j]
Amines	nt	≤10	-	nt	nt	1-napthylamine	1-10[h]

a. 210 micrograms extract mass used per test, except for alcohols test for EW83 extract.
b. For standard compound.
c. Poly and aralkyl mono-carboxylic acids.
d. 100 micrograms extract mass used.
e. 50 micrograms extract mass used.
f. Not tested; see text.
g. 5.25 mg extract used for test with methanol. P&VP fractions used a different reagent and benzylalcohol and 2-propanol as standards..
h. Test was negative for lower amount and positive for higher amount as 210μg aliquots.
i. 140 micrograms extract used.
j. Estimated from reference 16.

Table IV. Specific mass mutagenic activities of extracts of airborne particulate matter from SRM 1649 and Elizabeth NJ in the microsuspension procedure of the Salmonella/microcosm (Ames) assay.

Sample	Percent of Particle Mass[a]	TA-98 Revertants per microgram of extract[b] -S9	+S9
SRM 1649 DCM	5.7 ± 0.2	20.5 ± 6.1	13.2 ± 8.2
SRM 1649 ACE	2.0 ± 0.2	23.6 ± 15.4	20.8 ± 8.2
Elizabeth NJ ACE	20.5	5.9 ± 1.0	3.1 ± 0.4
P Fraction	10.3	7.0 ± 0.4[c]	5.8 ± 1.0[c]
VP Fraction	4.9	1.0 ± 0.2[c]	1.0 ± 0.06[c]
Blank		0.1	0.1

a. SRM 1649 particulate matter collected in filterbags and screened through a fine mesh sieve; Elizabeth particulate matter samples are $D_{50} \leq 15 \mu m$.

b. ± 95% confidence interval.

c. Determined in a different experiment than other values.

Table V. Specific mass mutagenic activities (revertants/μg extract) from SRM 1649 and Elizabeth NJ acetone extracts tested using the microsuspension assay with nitroreductase-deficient tester strains.

Sample	Specific Mass Mutagenic Activities		
	TA98[a]	TA98 NR[b]	TA98 1,8-DNP_6[c]
SRM 1649 ACE	25.3 ± 11.6[d]	8.4 ± 2.0	3.4 ± 2.0
Elizabeth NJ ACE	9.2 ± 3.0[d]	2.7 ± 0.6	0.8 ± 1.0

a. Same tester strain as used in table 4.
b. Nitroreductase-deficient tester strain.
c. Tester strain that insensitive to 1,8-dinitropyrene.
d. Separate experiment from that reported in Table 4.

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