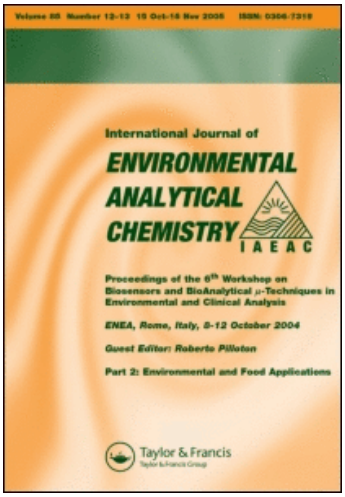

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The Identification of Polynuclear Aromatic Hydrocarbon (PAH) Derivatives in Mutagenic Fractions of Diesel Particulate Extracts†

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The soluble organic fraction (SOF) of particulate matter from diesel exhaust (from point sources, ambient air, etc.) contains hundreds of organic constituents. Normal-phase high pressure liquid chromatography (HPLC) has been used to separate the SOF into sub-fractions suitable for subsequent chemical analysis and bioassays. These fractions consist of non-polar(PAH), moderately polar (transition) and highly polar constituents. The non-polar fractions have been well characterized and consist of PAH and aliphatic hydrocarbons. The specific compounds present in the transition and polar fractions are for the most part unknown. This analytical information has been difficult to obtain since these compounds are highly labile, polar, of low volatility and in very low concentrations when compared to the bulk of material found in the SOF. Mutagenicity tests using the Ames Salmonella typhimurium assay indicate that the transition fraction accounts for most of the mutagenicity when compared to the non-polar (PAH) and polar fractions.

A variety of chromatographic and mass spectrometric techniques are described that have been used to determine the composition of the HPLC fractions. More than one hundred species have been identified in the transition fraction of diesel particulate matter using high

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resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS), HPLC and direct-probe high resolution mass spectrometry. It has been found that the transition fraction contains mostly PAH derivatives consisting of hydroxy, ketone, quinone, carboxaldehyde, acid anhydride and dihydroxy derivatives of PAH. Three nitro-PAH species have been tentatively identified and 1-nitropyrene positively identified in the transition fraction. The 1-nitropyrene was found to account for approximately 45% and 30% of the direct-acting mutagenicity observed for the transition fraction and total extract, respectively. The HPLC separation procedure was shown to give better than 95% recovery of the mass and mutagenic activity. The problem of PAH oxidation during the analytical procedures and possible effect on bioassay results are discussed.

INTRODUCTION

The chemical composition of particulate matter from diesel exhaust is very complex. It is likely that several hundred compounds are present.[1] The organic solvent extractable fraction is believed to contain the components of most environmental significance.

Because there are a large number of compounds present, various procedures are used to separate the soluble organic fraction (SOF) into subfractions containing compounds which have similar functional groups.[2–7] These groups consist of the acid, base and neutral fractions. The neutral fraction may be further separated by HPLC into nonpolar, moderately polar and highly polar fractions designated as the PAH, transition and oxygenate fractions, respectively.[3]

The Ames Salmonella mutagenesis bioassay (Ames test) has been used for determining the mutagenicity of the soluble organic fraction (SOF) of particulate samples collected from ambient air,[7–17] and power plant,[18] vehicle,[19–22] and cigarette smoke[23] emissions. Table I summarizes some of these data. Most particulate extracts give an Ames response (strain TA98, without enzyme activation: −S9), of between 500 and 5000 revertants/mg. These values can be compared to that for benzo (a) pyrene (45,000 revertants/mg with enzyme activation: +S9).

The possible health and environmental impact of exhaust particulates emitted by diesel engines [24–26] has received increasing attention recently. Investigations by EPA and others[3,7,16,19–21] using the Ames test have shown that most of the mutagenic activity in diesel particulate extracts is concentrated in fractions other than the PAH fraction.[20,27] Similar observations have also been made for ambient air particulate extracts.[12,15,16]

Prior to those studies, it was believed that the PAH were primarily responsible for mutagenic and possibly carcinogenic activity of particulates.[28–31]

Recent studies have shown that some PAH compounds can react readily with filter media,[32] nitrogen oxides,[33,34] sulfur oxides,[35] sulfuric

acid aerosols,[36] ozone[34–37] and gas-phase photochemical smog.[34] A number of compounds were tentatively identified when gas-phase (filtered) photochemical smog was drawn over benzo(a)pyrene(BaP) deposited on a glass fiber filter. Compounds identified included BaP-dihydrodiol(s), BaP-diphenol(s), BaP-phenol(s), BaP-quinones and what appear to be dicarbonyl compounds formed by ring-opening oxidations.[34] Some of the nitro-BaP derivatives were found to be direct acting mutagens.

The oxidation[37] of PAH has been studied under simulated conditions using ^{60}Co gamma radiation, 254 nm light and visible light. The products were found to be direct acting mutagens (−S9) toward various strains of Salmonella typhimurium. The mutagenicity of BaP increased from background to 7,200 and 6,240 rev/mg after exposure to ^{60}Co gamma radiation for 7 days and visible light for 18 days, respectively. Therefore the possibility exists that some of the products generated in these studies are responsible for the bioassay responses reported for the moderately and highly polar fractions of the organic extracts from particulate matter. However, the chemical identities of these substances are unknown.

Recently, a number of investigators have reported the limited identification of some PAH derivatives in HPLC fractions. Erickson, *et al.*[38] reported the identification of alkyl-9-fluorenones in a diesel particulate extract. Chaigneau *et al.*[39] found in soot a compound with a molecular weight corresponding to nitro-naphthalene and Rappaport, *et al.*[40] tentatively identified cyclopenteno (c, d) pyrene dicarboxylic acid anhydride in a highly mutagenic fraction of a diesel particulate extract.

Preliminary analytical studies from our laboratory[41] have shown that the transition fractions contain primarily oxygenated-PAH (oxy-PAH) compounds and the oxygenate fraction contains oxygenated PAH and oxygenated heterocyclic compounds. The purpose of this paper is to report an extensive study to identify the chemical constituents of diesel particulate extracts.

In this work, the Ames Salmonella typhimurium mutagenesis assay[42–45] was used to screen various fractions of particulate extracts separated by high performance liquid chromatography (HPLC). The fractions with the highest relative degree of activity were then analyzed using HPLC[46] gas chromatography/mass spectrometry (GC/MS) and high resolution mass spectrometric (HRMS) techniques.

Direct insertion probe distillation and GC/MS with acquisition of high accuracy data under conditions of moderate (1.5–2.0 K) and high (12–15 K) resolution modes of operation are used to analyze the fractions.[1] The probe distillation technique facilitates the analysis of those highly polar or more labile compounds not suitable for GC. The HRMS provides an accurate molecular formula which is used to help substantiate the identification.

The primary objective of this study is to determine the chemical composition of the transition fractions since they represent more than 50% of the Ames mutagenicity in the soluble organic fraction (SOF) (Table II). Preliminary results are reported on a subfraction β of the PAH fraction and the oxygenate fraction, and further results will be presented in future publications.

Another objective of this study is to determine the possibile conversion of PAH and oxy-PAH to other species during the analytical procedures. Many of the PAH compounds are known to be air sensitive and oxidize readily. For instance, it has been reported in the literature that 9,10 dihydrophenanthrenes are very air sensitive and rapidly oxidize to the 9, 10 phenanthrene quinone.[47]

EXPERIMENTAL

Particulate collection-extraction

Light duty diesel exhaust particulate samples were collected on T60A20 Pallaflex filters (Teflon impregnated glass fiber filters) using an exhaust dilution tube. The two different samples described in this study are designated as NI-1 and OL-1 in Table II. The particulates were extracted with methylene chloride ($MeCl_2$) using soxhlet extraction techniques. Extracts in methylene chloride ($\approx$1 mg/ml) were stored at dry ice temperatures until use. Analytical procedures were performed in subdued room incandescent light to avoid possible photochemical modifications.

TABLE I
Ames Salmonella Typhimurium mutagenicity for some environmental particulate solvent extracts

Source	Mutagenicity (Revertants/mg)[a]
Non-Catalyst Gasoline Engines[27] (Average of Five)	900
Four Cylinder Diesel Engine[19]	2,000
Truck Diesel (4-Stroke)[27]	4,300
Cigarette Smoke Condensate[23]	1,100
New York City (Winter)[16]	1,300
New York City (Summer)[16]	1,600
Residential Area (Japan)[10]	44–1800
Industrial Area (Japan)[10]	290–2200
Power Plant[18]	2,600
Coke Mill[10]	1,200

[a]TA98 Strain Without S9 Activation

Solvents and standards

All the solvents were "distilled in glass" UV grade obtained from Burdick and Jackson. PAH compounds were obtained from Aldrich, Analabs, Inc., and Columbia Organics Chemical Company. PAH derivatives were obtained from a number of sources including other research laboratories, commercial sources and some were synthesized in-house. Approximately 125 PAH-derivatives are currently available.

High performance liquid chromatography (HPLC) fractionation

Fractionation of the extracts was performed with a Waters HPLC system which consists of two Model 6000 pumps, a Model U6K injector, a model 660 gradient programmer, a Model 440 dual micro UV detector and a Schoeffel Model FS-970 LC spectrofluorometer.

The analytical HPLC procedure utilized both low and high resolution columns for fractionation of the extracts. The low resolution column was 3 mm × 30 cm stainless steel, packed with Biosil-A (Bio-Rad (20–44 μ)) preceded by a 4 mm × 5 cm stainless steel precolumn packed with Biosil-A. The fluorescence detector was used with setting at $\lambda_{EX} = 313$ nm and $\lambda_{EM} \geqq 418$ nm. The solvent flow was 1 ml/min. Acetonitrile was injected in 2–3 ml slugs to clean the column and establish a constant δ, blank. This was followed by an 8 minute flush with a 5% methylene chloride($MeCl_2$) in *n*-hexane solvent mixture. A 25 μl sample of extract in $MeCl_2$ was injected into the column using 5% $MeCl_2$ in hexane as an eluant. After 17 minutes under isocratic conditions a programmed gradient of 5% min $MeCl_2$ was started. At 36 minutes the solvent was 100% $MeCl_2$. The eluant was then held at 100% $MeCl_2$ for 17 minutes and 2.0 ml of acetonitrile was injected to elute the δ_1 peak. After the δ_1 peak was eluted, 2.0 ml of methanol was injected to elute the δ_2 peak. After equilibrium a reverse programmed gradient (10% hexane/min) down to 5% $MeCl_2$ was started in preparation for the next analysis.

The high resolution technique utilized a Waters normal-phase radial compression column (RCSS, Radial-PAK B). The mobile phase solvents used were hexane, $MeCl_2$ and acetonitrile. In some of the analyses, chloroform was used instead of $MeCl_2$ in the above solvent system. The two systems gave similar elution order for all of the compounds tested in this study.

During analysis, the sample was injected while the column was eluted with *n*-hexane. After 20 minutes the eluant was programmed from *n*-hexane to $MeCl_2$ using a solvent gradient preset in the programmer (curve 9, a non-linear gradient preset in the solvent programmer) with $\approx$5% $MeCl_2$ up to 10 minutes and then nearly exponential progression to

100% $MeCl_2$ in the second half of the gradient period. The solvent was held at 100% $MeCl_2$ for six minutes after the end of the gradient. It was then switched from $MeCl_2$ to acetonitrile through a pneumatically controlled 4-way Valco valve installed on the pump solvent supply line. The column was then eluted isocratically with acetonitrile for 10 minutes before it was reverse programmed to the original *n*-hexane elution conditions.

A 8 mm × 25 cm Michel-Miller glass prep column was used to separate adequate size fractions for Ames testing and mass spectrometric analysis. A 9 mm × 50 cm Magnum-9 (Whatman) reverse phase column was used to separate the β peak of the NI-1 sample (Figure 1) into thirty fractions using methyl alcohol at 4 ml/min as an eluant. The peak elution profile for the analytical column (Figure 1) and the preparative column were similar except that somewhat poorer baseline resolution for the various fractions was noted in the case of the preparative column.

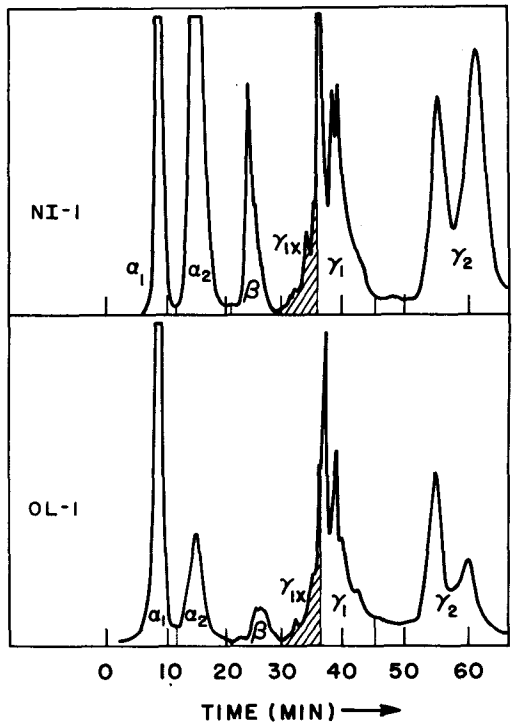


FIGURE 1 HPLC fractionation for diesel particulate extract samples OL-1 and NI-1 utilizing a 3 mm × 30 cm, normal phase Biosil A (20–44 μ) silica column with fluorescence detection at $\lambda_{ex} = 313$ nm; $\lambda_{em} = \geqq 418$ nm.

High resolution mass spectrometry

High resolution mass spectral (HRMS) analyses were performed on a Vacuum Generators, Inc. Micromass Zab-2F operated at 11,000 to 15,000 resolving power in the electron impact (70 ev) mode. Samples were injected into the mass spectrometer using a direct insertion probe inlet system. The MS source was kept at 240°C and the probe temperature programmed from 20°C to 300°C.

Gas chromatography/mass spectrometry

GC/MS analyses were performed on a VG-Micromass MM-16 mass spectrometer interfaced with a Varian 1400 GC using a single stage glass jet separator. Six-foot packed columns consisting of either SP 2250 (1%) on Supelcoport or Dexsil 300 (2%) on Ultrabond C were used for the GC/MS analysis of the HPLC transition (γ_1, γ_2) and oxygenate (δ) fractions. The Dexsil 300 column was programmed from 80–350°C at 6°C/min and the SP2250 column programmed from 80–320°C at 4°C/min.

The α_1, α_2 fractions were combined and the aliphatic hydrocarbons selectively removed using the procedure developed by Grimmer[48] including solvent/solvent partitioning and Sephadex LH-20 column pre-separations to remove aliphatics as described in previous work[36] from this laboratory. GC/MS analysis was performed using a 25 m Dexsil 300 capillary column.

Mutagenicity testing

Fractions collected from the low resolution HPLC technique were tested using histidine-requiring strains of Salmonella typhimurium-TA98. The solvent was removed and the residue dissolved in DMSO. Liver microsomal fraction (+S9) was used for activation.[42] Aliquots of each fraction were taken so that there were at least three points within the linear region of the dose-response plot. The mutagenicity of each fraction in revertants/mg(A_n) and of the total extract(A_T) was determined from the least squares linear fit of the dose-response plot. 1-nitro fluorene was used as a standard for the direct acting mutagenicity studies. Strain TA 98 was chosen because of its high sensitivity for mutagenic assays of diesel particulate extracts.

RESULTS AND DISCUSSION

HPLC fractionation

Open column liquid chromatography separation has been the most widely used method for the fractionation of complex environmental particulate

samples according to chemical functionalities. The application of HPLC as a means of direct fractionation of the particulate extracts has recently begun to gain popularity due to its reproducibility and speed. HPLC fractionation of diesel particulate extracts has recently been reported[20] and is similar to that described in the Experimental Section for the low resolution HPLC analysis. Figure 1 shows the elution profile using fluorescence detection for the NI-1 and OL-1 diesel particulate extracts using this procedure. The neutral components of the extract are divided into non-polar PAH: α_1, α_2, β), moderately polar (transition: γ_1, γ_2) and polar subfractions (oxygenates: δ_1 and δ_2; not shown in the figure).

The diesel extracts gave qualitatively similar chromatograms. However, the intensities of the α_2, β and γ_2 peaks were greater for sample NI-1 than OL-1. HPLC analysis of solvent extracts from ambient air particulates have been found to show qualitatively similar chromatograms (32, 41).

Each fraction eluted from the HPLC column was collected at the times shown in Figure 1. Table II gives the percentage of material eluted in each fraction. Nearly 100% of the material injected into the HPLC was recovered using this procedure.

In this work, the low resolution HPLC technique has been modified such that the fractions are separated with better resolution. Figure 2

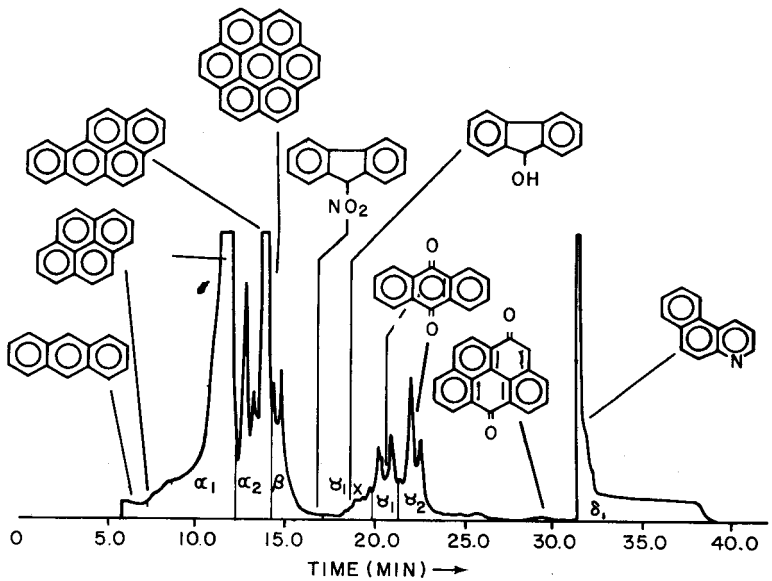


FIGURE 2 HPLC fractionation and elution of some standards (see Table III) for a diesel particulate extract (NI-1) utilizing an RCM high resolution normal phase silica column (Radial-PAK B) with fluorescence detection at $\lambda_{ex} = 313$ nm; $\lambda_{em} = 418$ nm.

TABLE II

Ames Salmonella typhimurium mutagenicity (TA98), fluorescence response and mass for various HPLC fractions of two diesel particulate extracts

HPLC fraction	Sample NI-1[a]						Sample OL-1[a]					
	Ames activity[b] Absolute (A_n)		Ames activity[b] Percentage (R_n)		Mass Wgt. %	Fluorescence Response (%)	Ames activity[b] Absolute (A_n)		Ames activity[b] Percentage (R_n)		Mass Wgt. %	Fluorescence Response (%)
	−S9	+S9	−S9	+S9			−S9[d]	+S9	−S9	+S9		
α_1						10.4						22.2
	<50	—	<1	20	57.4		<25	250	<2	16	53.0	
α_2						20.4						6.1
β	87,500 (<3,000)	—	24 (<1)[c]	21	1.3	5.8	2,000	4,800	5	10	1.7	2.2
γ_1	38,800	—	43	24	4.3	20.2	6,100	3,500	64	33	6.9	25.3
γ_2	23,800	—	15	13	5.0	23.6	2,500	4,500	9	13	2.3	18.3
δ_1	7,200	—	17	22	16.0	19.6	890	850	23	19	16.1	25.9
δ_2	—	—	—	—	14.7	—	—	—	—	—	16.5	—
Total (fractions)	—	—	—	—	98.7	100.0	655	721	103	91	96.5	100.0
Total (extract)	12,500	—	—	—	100.0	—	630	790	100	100	100.0	—

[a]See Figure 1 for HPLC profiles.
[b]Absolute Ames activity given in revertants/mg.
[c]Value in parentheses represents that mutagenicity found when the β fraction was carefully separated from the δ_1 fraction (i.e. the β fraction was cut prior to the time at which nitro naphthalene elutes) and assayed within 24 hours after collection.
[d]1-nitrofluorene std. gave approximately 150,000 revertants/mg.

shows the HPLC fractionation of diesel extract NI-1 utilizing the high resolution RCM column described in the Experimental Section. The baseline separation between the two fractions is greatly improved which allows collection of the transition fraction without contamination from the PAH fraction. The elution times of several standards are shown in Table III. Because there are a large number of compounds present in the particulate extracts when compared to the limited resolving power of HPLC, the determination of the detailed chemical composition of these fractions requires the use of spectroscopic techniques such as mass

TABLE III

HPLC retention times of some PAH and PAH derivatives using an RCM high resolution normal phase silica column (radial peak B)[a]

Compound name	MW	Retention time (detector)[b] (min)	
		UV	Fluorescence
α_1, α_2 Fraction (0–13.5 min)			
Naphthalene	128.063	4.3	—
Anthracene	178.078	6.6	—
Pyrene	202.078	6.7	6.8
Fluoranthene	202.078	8.1	—
Xanthene	182.073	9.5	—
Benzo(a)pyrene	252.094	12.3	—
Benzo(e)pyrene	252.094	12.4	12.3
Perylene	252.094	12.5	—
1,2-Benzo(k)fluoranthene	252.094	12.6	—
Benzo(g, h, i)perylene	276.094	12.7	12.6
Chrysene	228.094	12.9	12.9
β Fraction (13.5–17.0 min)			
Coronene	300.094	13.7	13.7
β-19 peak (see Figure 2)		14.2	—
1, 2, 4, 5 Dibenzopyrene	302.109	14.4	14.5
1, 2, 3, 4 Dibenzanthracene	278.109	14.5	—
Hydroxybenzyl Alcohol	124.052	15.0	14.8
2-Nitro Fluorene	211.063	17.0	17.0
γ_1 Fraction (17.0–21.5 min)			
2-Nitro Naphthalene	173.048	17.5	—
9-Hydroxy Fluorene	182.073	—	18.3
1-Nitro Pyrene	247.063	19.5	—
6-Nitro Benzo(a)pyrene	297.079	20.4	—
2-Hydroxy-9-Fluorenone	196.052	20.4	20.4
7-Benzo(a)Anthracene Carboxaldehyde	256.089	20.7	20.7
9-Anthracene Carboxaldehyde	206.073	20.7	20.7
9-Fluorenone	180.057	21.0	21.0
1-Pyrene Carboxaldehyde	230.073	21.7	21.7

TABLE III (Continued)

Compound name	MW	Retention time (detector)[b] (min)	
		UV	Fluorescence
γ_2 Fraction (21.5–25.0 min)			
9,10 Anthracene Quinone	208.052	22.0	—
2,7 Dinitro Fluorene	256.048	22.4	—
3,8 Pyrene Quinone	232.052	22.5	23.0
5,6 Chrysene Quinone	258.068	24.2	—
7 Hydroxy Benzo(a)pyrene	268.089	24.4	24.7
9-Anthrone	194.073	25.3	—
γ_3 Fraction (25.5–30.5 min)			
1,6-Benzo(a)pyrene Quinone	282.068	28.4	—
9-Xanthone	198.068	29.1	—
7-Benzoanthrone	244.089	30.1	—
δ_1 Fraction (30.5–40.0 min)			
Acridine	179.073	31.7	31.6
Benzo(c)cinnoline	180.069	31.9	31.8
7-Benzanthracene Carboxylic Acid	272.079	30–32	—
5,6-Benzoquinoline	179.073	32.0	32.0

[a]See Figure 2 for fraction designation. γ_{1x} fraction elutes from 16.8–20.0 minutes. See Figure 1 for designation of similar fractions obtained using the Biosil column.

[b]Retention times are accurate to within ±0.5 min. Detectors are in series.

spectrometry. Nevertheless, the elution of standards through the HPLC can be used to determine the general classes of compounds that may be found in each fraction. PAH (2–4 rings) and PAH (4–6 rings) elute in the α_1 and α_2 fractions, respectively. PAH (6–8 rings), hydroxy benzenes, and nitro-PAH (2 rings) elute in the β region. Nitro PAH (2–6 rings), hydroxy PAH (3–4 rings). PAH carboxaldehydes (3–4 rings), PAH quinones (3 rings) and ketones (2–3 rings) elute in the γ_1 region. PAH quinones (3–5 rings), hydroxy PAH (5–7 rings), PAH ketones (3–4 rings), dihydroxy PAH (3–5 rings) and dinitro PAH (3 rings) elute in the γ_2 region. PAH quinones (>6 rings), PAH carboxylic acids (>3 rings) and nitrogen containing heterocyclics elute in the δ_1 fraction.

An advantage of the HPLC procedure depicted by Figure 2 is the excellent baseline resolution between the (α_1, α_2, β), (γ_1, γ_2) and δ regions. Poor baseline separation between the ($\alpha_1, \alpha_2, \beta$) and ($\gamma_1, \gamma_2$) regions results in contamination of the (γ_1, γ_2) region by aliphatics and PAH compounds, thus making analysis much more difficult.

There are several noticeable differences between the low and high resolution HPLC elution patterns. The elution of the β peak occurs

approximately midway between the α_2 and γ_1 peaks in the low resolution procedure (Figure 1) and right after the α_2 peak in the high resolution procedure (Figure 2). A subfraction of the β peak (B #19) was collected from the low resolution column and re-injected into the high resolution column. The B #19 material was eluted at 14.2 min, within the β region depicted in Figure 2. This result indicates that the two β peaks eluted by these two different HPLC procedures contain similar chemical constituents. Data presented in this paper will show that the β peak consists primarily of high molecular weight PAH and alkyl-substituted PAH.

Mutagenicity testing

Fractions collected from the HPLC technique were tested for mutagenic activity as described in the Experimental Section. Table II gives the results of these tests. Unfortunately, the mass of material used for the Ames assay on sample NI-1 was not determined. However, the percentage of mutagenicity exhibited by each fraction (R_n) could be determined by Equation 1.

$$R_n = \frac{(A_v)(V_n)}{\sum_{n=1}^{n=x} (A_v)(V_n)} \times 100 \tag{1}$$

where

A_v = mutagenicity in revertants/μl for each fraction.
V_n = total volume (μl) collected for fraction n
x = total number of fractions

The mutagenicity of each fraction (A_n) in revertants/mg was calculated from Equation 2 using the assumption that the mutagenicity of the total extract was equivalent to the summation of the fraction mutagenicity (this assumption was found to be valid for sample OL-1).

$$A_n = \frac{(A_T)(R_n)(M_n)}{\sum_{n=1}^{n=x} (R_n)(M_n)} \tag{2}$$

where

A_T = mutagenicity (revertants/mg) for the total extract
R_n = percentage of mutagenicity exhibited by fraction n as calculated from Equation 1
M_n = mass(mg) for fraction n
x = total number of fractions.

The mutagenicity of each fraction (A_n) and the total extract (A_T) for

sample OL-1 was experimentally determined. From these results, the percentage of Ames activity recovered from the HPLC (A_{HPLC}) can be calculated as given by equation 3.

$$A_{HPLC} = \frac{\sum_{n=1}^{n=x} A_n(M_n/M_T)}{A_T} \times 100 \quad (3)$$

where

A_n = mutagenicity (revertants/mg)
M_n = mass(mg) of fraction n collected from the HPLC
M_T = Mass(mg) of total extract injected into the HPLC
A_T = mutagenicity(revertants/mg) of the total extract

Using this equation, it was found that 103% of the direct acting mutagenicity and 91% of the indirect acting mutagenicity was recovered from the HPLC.

The mutagenicity for the δ_2 fraction was not determined. However, it probably represents less than 10% of the total mutagenicity since the other fractions account for more than 90% of the total extract mutagenicity.

It would be of value if a simple HPLC fluorescence measurement could be made that correlated with Ames mutagenicity. This type of study has been undertaken in the past and limited results showed a good correlation between these two parameters (20). Equivalent quantities of each extract were injected into the HPLC (32 μg). The fluorescence responce of each fraction was integrated and the percentage of fluorescence exhibited by each fraction was calculated as given in Table II. The most significant difference between the HPLC fluorescence profiles of these two samples is the relative intensities of the α_2 and β peaks (Figure 1). It is possible that the increased fluorescence as exhibited by these two HPLC fractions is related to the observed increase in mutagenic activity. However, from Table II it is seen that all the fractions for sample NI-1 show higher absolute activity than that observed for sample OL-1. Therefore, there does not appear to be a relationship between fluorescence and mutagenicity for these two samples.

The α_1, α_2 fractions for extract NI-1 and OL-1 represented less than 1–2% of the mutagenicity for the total extract without S9 activation which indicate that there are no significant concentrations of direct acting mutagens. The indirect acting mutagenicity (16–20%) was significant considering the fact that 85–90% of this fractions mass consisted of non-mutagenic aliphatic hydrocarbons, alkyl benzenes and alkyl naphthalenes.

The β fraction for sample NI-1 was found to exhibit the highest level of indirect acting mutagenicity (87,500 revertants/mg) for any of the fractions (Table II). It was found (Table III) that mutagenic species such as nitro naphthalene and nitro fluorene were eluted very near the point at which the β and γ_1 fractions were separated for biological and chemical assays. Therefore, care was taken to insure that the β fraction was cut prior to the time at which these species eluted. In addition, evidence is presented in this paper that shows that the chemical species present in the β fraction readily undergoes oxidation during storage after separation by HPLC. Therefore, another experiment was undertaken to carefully separate the β fraction and undertake the bioassay within 24 hours after collection. In this case, it was found that the β fraction exhibited no detectable direct acting mutagenicity ($<$3,000 revertants/mg). The β fraction for sample OL-1 was analyzed in the same manner and 2,000 revertants/mg of direct acting mutagenicity was measured. A problem encountered with bioassay of the β fraction was the small quantity of material available for testing. Thus, in order to obtain sufficient material, it was necessary to use larger HPLC columns which resulted in lower separation resolution. Therefore, it was much more difficult to assure that a portion of the nitro PAH species were not collected in the β fraction.

The indirect acting mutagenicity of the β fraction was the highest observed for any fraction and the γ_1 and γ_2 fractions represented more than 50% of the direct acting mutagenicity. Therefore, these three fractions were chosen for detailed chemical analysis.

Mass spectrometric analysis

Several HPLC fractions for both diesel extracts were analyzed using a combination of direct probe high resolution mass spectrometry (HRMS) and GC/MS. High accuracy data may be acquired in low or high resolution modes as described previously.[1] The probe technique facilitates the analysis of the highly polar or labile compounds not suitable for GC. In addition, HRMS is used to provide an accurate molecular formula to help substantiate the GC/MS identifications.

It was found that at least 12,000 resolution (10% valley) was necessary to resolve mass fragments adequately. For instance, at nominal mass 208, two empirical formulas are possible for PAH Oxygenates; $C_{14}H_8O_2$ (m/z: 208.0532) and $C_{15}H_{12}O$ (m/z: 208.0872). The possible PAH-oxy structures with the empirical formulas $C_{14}H_8O_2$ include (anthracene and phenanthrene) quinones, fluorenone carboxaldehydes and cyclopentenone napthalene carboxaldehydes. Possible PAH-oxy structures with the

empirical formula $C_{15}H_{12}O$ include methyl (anthrones and phenanthrones), dimethyl fluorenones, methyl fluorene carboxaldehydes, dimethyl cyclopentenonaphthones, dimethyl cyclopentenone naphthalenes and methyl hydroxy (anthracene and phenanthrenes). In all, there are well over 200 isomers of these species which makes positive identification of isomers a nearly impossible task. However, each of these compound classes give distinct spectra which may be used to distinguish one class from another.

Data obtained from direct probe distillation were compared with the GC/MS analysis. The two techniques were used in a comparative way. Identification of components by GC/MS was undertaken and the relative abundance of each component normalized to the most abundant compound (9-fluorenone and naphthalene dicarboxylic acid anhydride for fractions γ_1, and γ_2, respectively). The same procedure was undertaken for the direct probe HRMS analysis. The difference between the relative abundance for a particular species as given by HRMS and GC/MS will be positive if there are compounds that are not eluted or separated in the GC/MS procedure. The difference will be negative if more species are eluted from the GC/MS than from the HRMS technique. To date, significant negative differences have not been encountered. An equivalent relative abundance between the HRMS and GC/MS indicates that all possible components represented by a particular accurate mass have been accounted for in the GC/MS analysis.

High ($\pm$0.003 AMU) or nominal ($\pm$0.1 AMU) accuracy mass chromatograms were generated for the three or four most intense ions of each compound. Very often a large number of isomers may be possible for each oxygenated or nitrated PAH derivative. For instance, methyl (phenanthrene or anthracene) carboxaldehyde has 72 possible isomers. Many of the higher molecular weight PAH derivatives have considerably more isomers, making absolute identification nearly impossible. Mass spectra on a large number of standard compounds were studied in order to determine the chemical composition of species present. Very often, a series of isomers for substituted PAH give similar spectra but can be used to establish the presence of a class of compounds.

All the chemical analytical data presented in this paper is for sample OL-1 except for the analysis of the β fraction which was derived from sample NI-1.

Analysis of the α_1, α_2 fractions

In sample OL-1, the aliphatic hydrocarbons were selectively removed and analyzed by capillary column GC/MS as described in the Experimental

Section. Table IV gives the relative abundance of PAH species identified in these fractions. PAH were identified in the MW range from 173 to 302 and methylated PAH were the most abundant PAH species. The data for this fraction was acquired for the purpose of comparing the relative abundance of the PAH species with that of the PAH derivatives. The composition of this fraction is constant with previous analyses as determined by a number of other investigators.[6,29,30,36,48]

Analysis of the β fraction

The β fraction (sample NI-1) was divided into thirty additional subfractions using a reverse phase column as described in the Experimental Section. The β fraction for the NI-1 extract was chosen because this sample exhibited an unusually intense HPLC fluorescence peak as compared to that obtained for a large number of other diesel extracts. Direct probe HRMS analysis was used to identify components on a relative quantitative basis in fractions #24–30 (Figure 3) and on a qualitative basis for the other fractions. The early eluting fractions (#1–10) could contain some nitro-PAH species. However, it is expected that they would have eluted (if present) before the first fraction was collected, since the more polar species are eluted first when using the reverse phase HPLC separation. The compounds identified in fractions #24–30 and their relative abundance in each fraction are listed in Table V and illustrated in Figure 3. The most abundant species in this fraction has a mass of 300.094 ($C_{24}H_{12}$), the mass spectrum of which matches very closely with that of coronene. Cyclopenteno dibenzopyrenes also have a molecular mass of 300.094 but can be distinguished from the coronene by their abundant ($\approx 40\%$) M−1 and M−2 ions relative to the parent ion. The abundance of these two ions from coronene were less than 10% (relative to M^+).

There appears to be two possible compounds of molecular weight 302.106 based upon the variation in relative abundance of the $(C_{24}H_{14})^+$ ion from fraction #24–30, as shown in Figure 3 (open boxes). These species have been identified as two isomers of dibenzopyrene.

A major component at mass 290.109 ($C_{23}H_{14}$) is tentatively identified as methyl anthanthrene or isomers. Cyclopentacoronene or cyclopentenodibenzopyrene ($C_{26}H_{14}$) and anthanthrene or isomers ($C_{22}H_{12}$) were identified. A low relative concentration of hydroxy coronene was found but we propose that this specie was probably formed during the analytical process or storage since hydroxy coronene would not be eluted in this fraction during the HPLC fractionation process (see Table III).

TABLE IV

Relative abundance of PAH species identified by GC/MS (25 m Dexsil 300 capillary column) and HRMS for the PAH (α_1, α_2) HPLC fractions of a diesel particulate extract (OL-1)

Compounds	Formula	Mass[a]	Relative[b] abundance
Phenanthrene and Anthracene	$C_{14}H_{10}$	178.078	0.27
Methyl (Penanthrenes and Anthracenes)	$C_{15}H_{12}$	192.094	0.54
Fluoranthene	$C_{16}H_{10}$	202.078	0.41
Pyrene	$C_{16}H_{10}$	202.078	0.41
Dihydro (Fluoranthenes and Pyrenes)	$C_{16}H_{12}$	204.094	0.09
Dimethyl (Phenanthrenes and Anthracenes)	$C_{16}H_{14}$	206.110	1.00
Benzofluorenes	$C_{17}H_{12}$	216.094	0.67
Methyl (Fluoranthenes and Pyrenes)	$C_{17}H_{12}$	216.094	0.55
Benzylnaphthalenes	$C_{17}H_{14}$	218.110	0.18
Trimethyl (Phenanthrenes or Anthracenes)	$C_{17}H_{16}$	220.126	0.37
Cyclopentapyrene	$C_{18}H_{10}$	226.078	0.47
Benzo(a) Anthracene(BaA), Chrysene, and Triphenylene	$C_{18}H_{12}$	228.094	0.16
Diphenylbenzene	$C_{18}H_{14}$	230.110	0.11
Methyl (BaA, Chrysene and Triphenylene)	$C_{19}H_{14}$	242,110	0.42
Benzofluoranthenes	$C_{20}H_{12}$	252.094	0.33
Benzo(e) Pyrene(BeP), Benzo(a) Pyrene(BaP) and Perylene	$C_{20}H_{12}$	252.094	0.48
Methyl Benzofluoranthenes	$C_{21}H_{14}$	266.110	0.13
Methyl (BeP, BaP, Perylene)	$C_{21}H_{14}$	266.110	0.16
Benzo(g, h, i) Perylene and Anthanthrene	$C_{22}H_{12}$	276.094	0.25
Coronene	$C_{24}H_{12}$	300.094	0.06
Dibenzopyrenes	$C_{24}H_{14}$	302.110	0.02

[a]Measured to within $\pm$ 10 ppm at 12K resolution.
[b]Abundance relative to dimethyl (phenanthrenes and anthracenes).

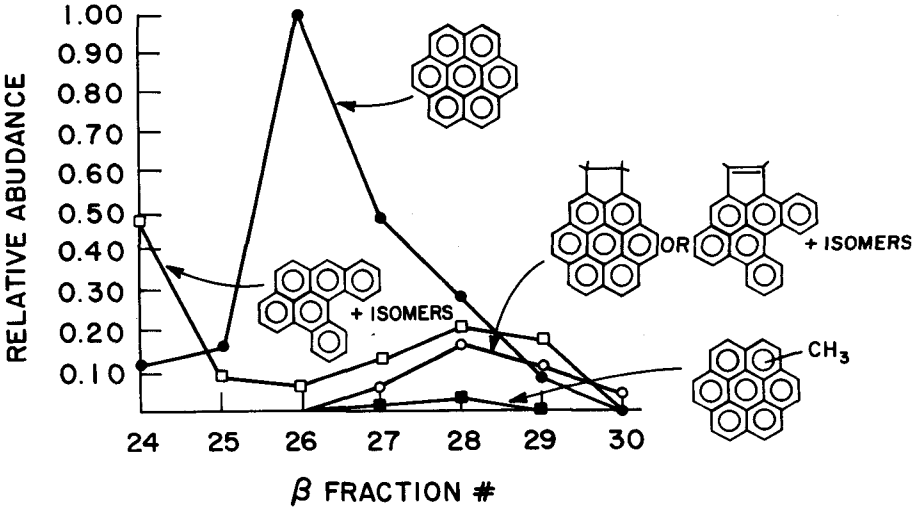


FIGURE 3 Relative abundance of PAH species identified by high resolution mass spectrometry for the β-HPLC fractions #24–30 of sample NI-1.

Fractions #1–23 were found to contain PAH species (MW 206–276) and in some cases the oxidation products of these PAH were present. The oxygenated derivatives of the PAH probably were formed during storage or the analytical procedure since they could not have eluted in the β fraction (see Table III). The susceptibility of individual PAH to oxidation can be estimated by comparing the abundance of the oxy-PAH to that of the parent PAH. Based upon this assumption, it appears that benzo(a) anthracene (or isomers) was one of the most easily oxidizable species present in fractions #1–14. The most abundant PAH species in selected fractions were as follows:

#3-dimethyl (anthracenes or phenanthrenes)
#5-cyclopentapyrene
#8-diphenylbenzene
#9-benzo(a) anthracene + isomers
#11-methyl diphenylbenzene
#12-methyl benzo(a) anthracene
#14-dihydro (benzo(a) pyrene + isomers)

Analysis of the γ_1, γ_2 fractions

Figures 4a, 4b and 5 give the computer reconstructed total ion current chromatograms for the GC/MS analysis of fractions γ_1 and γ_2, respectively

TABLE V
Chemical composition of the β–HPLC fractions of a diesel particulate extract (NI-1)

Compound	Mass		Relative abundance (fraction #)						
	Found	Theory	24	25	26	27	28	29	30
Anthanthrene or Isomers ($C_{22}H_{12}$)	276.097	276.094	0.181	0.009	—	—	—	—	—
Methyl Anthanthrene or Isomers ($C_{23}H_{14}$)	290.108	290.109	0.362	0.269	—	—	—	—	—
Coronene ($C_{24}H_{12}$)	300.092	300.094	0.121	0.159	1.000	0.480	0.293	0.073	—
Dibenzopyrene ($C_{24}H_{14}$)-2 Isomers	302.106	302.109	0.481	0.097	0.068	0.128	0.202	0.174	—
Methyl Coronene ($C_{25}H_{14}$)	314.109	314.109	—	—	—	0.010	0.028	—	—
Hydroxy Coronene ($C_{24}H_{12}0$)	316.091	316.089	0.034	0.042	0.049	0.051	0.032	—	—
Cyclopentacoronene or Cyclopentenodibenzopyrene ($C_{26}H_{14}$)	326.111	326.110	—	—	—	0.053	0.162	0.109	0.043

(sample OL-1). The compounds identified in these analyses are given in Tables (VI, VII) and (VIII, IX) for fractions γ_1 and γ_2, respectively. The data in Tables VII and IX represent a comparison of the relative abundance of PAH species identified by direct probe HRMS and GC/MS.

It was found that 9-fluorenone and naphthalic acid anhydride were the most abundant compounds in the γ_1 and γ_2 fractions, respectively. The

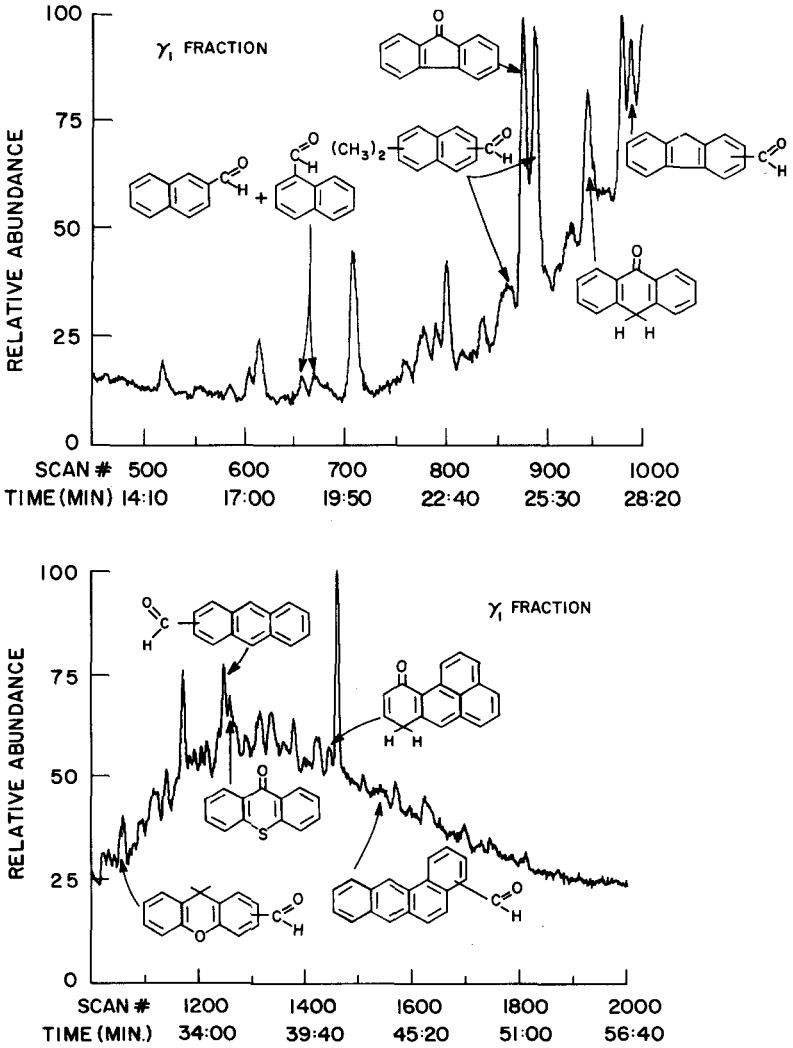


FIGURE 4 Total Ion Chromatograms for the GC/MS analysis of the γ_1 HPLC fraction for diesel particulate extract OL-1 utilizing a 6′ SP2250 column.

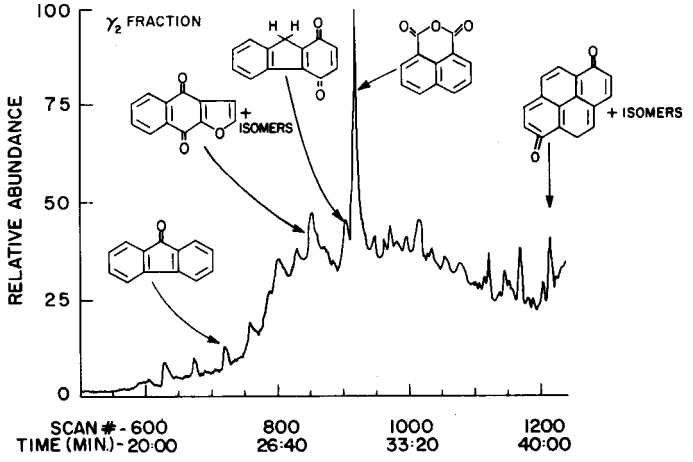


FIGURE 5 Total ion chromatogram for the GC/MS analysis of the γ_2 HPLC fraction of diesel particulate extract OL-1 utilizing a 6' Dexsil 300 column.

relative abundances of the other species are calculated with respect to these compounds using the sum of the integrated total ion current intensities for the parent ion and the two most abundant fragments. The relative abundance for each class (e.g. ketones, quinones) of the PAH-derivatives was determined from the data tabulated in Tables VI-IX and is discussed in the Summary and Conclusions Section.

Approximately 60 wgt% of the total material present in these two fractions are oxygenated derivatives of PAH as estimated by integrated total ion current abundances. The only heterocyclics found in these fractions are some oxidation products of thioxanthene and xanthene. The remainder of the γ_1 and γ_2 fractions mostly consist of aliphatic hydrocarbons and phthalate esters. These species were present as contaminants and arose from a number of sources during the HPLC fractionation and concentration procedures including the solvents, injection port septum and build up and subsequent bleed of these materials from the normal phase column. PAH derivatives identified including hydroxy, ketone, carboxaldehyde, quinone, dihydroxy, acid anhydride and nitro derivatives of PAH. Some details concerning MS identification of compounds are presented in the next section. A future publication describing the mass spectral interpretation of over 100 oxygenated and nitrated PAH standard compounds is in preparation.[49]

TABLE VI

Relative abundance of chemical species identified by GC/MS (SP 2250 column) in the transition (γ_1) HPLC fraction of a diesel particulate extract-OL-1[1]

Compound	Formula	Mass[2]	Scan #	Relative abundance
2-Naphthalene Carboxaldehyde	$C_{11}H_8O$	156.061	660	0.035
1-Naphthalene Carboxaldehyde	$C_{11}H_8O$	156.061	673	0.059
Methyl Naphthalene Carboxaldehyde	$C_{12}H_{10}O$	170.073	779	0.062
Naphthalene Acetaldehyde	$C_{12}H_{10}O$	170.073	789	0.073
Naphthalene Acetaldehyde	$C_{12}H_{10}O$	170.073	795	0.015
Dimethyl Naphthalene Carboxaldehyde	$C_{12}H_8O_2$	184.052	868	0.098
9-Fluorenone	$C_{13}H_8O$	180.058	881	1.000
Naphthalene Dicarboxaldehyde	$C_{12}H_8O_2$	184.052	894	0.140
Phenanthrene or Anthracene	$C_{14}H_{10}$	178.078	895	0.260
Naphthalene Dicarboxaldehyde	$C_{12}H_8O_2$	184.052	925	0.047
Phenanthrone or Anthrone	$C_{14}H_{10}O$	194.073	941	0.110
Naphthalene Dicarboxaldehyde	$C_{12}H_8O_2$	184.052	943	0.028
9-Anthrone	$C_{14}H_{10}O$	194.073	947	0.040
Trimethyl Naphthalene Carboxaldehyde	$C_{14}H_{14}O$	198.104	950	0.061
Phenanthrone or Anthrone	$C_{14}H_{10}O$	194.073	961	0.197
Trimethylnaphthalene Carboxaldehyde	$C_{14}H_{14}O$	198.104	965	0.062
Fluorene Carboxaldehyde	$C_{14}H_{10}O$	194.073	989	0.176
Methyl (Anthracene or Phenanthrene)	$C_{15}H_{12}$	192.094	991	0.128
Methyl (Anthracene or Phenanthrene)	$C_{15}H_{12}$	192.094	998	0.168
Fluorene Carboxaldehyde	$C_{14}H_{10}O$	194.073	1000	0.176
9-Xanthone	$C_{13}H_8O_2$	196.052	1004	0.039
Trimethyl Naphthalene Carboxaldehyde	$C_{14}H_{14}O$	198.104	1010	0.031
Fluorene Carboxaldehyde	$C_{14}H_{10}O$	194.073	1021	0.134
Methyl (Anthracene or Phenanthrene)	$C_{15}H_{12}$	192.094	1022	0.136

Trimethyl Naphthalene Carboxaldehyde	$C_{14}H_{14}O$	198.104	1040	0.030
Methyl 9-Anthrone	$C_{15}H_{12}O$	208.089	1057	0.241
Methyl (Anthrone or Phenanthrone)	$C_{15}H_{12}O$	208.089	1077	0.144
Dimethyl (Anthracene or Phenanthrene)	$C_{16}H_{14}$	206.109	1090	0.121
9, 10 Phenanthrene Quinone	$C_{14}H_{10}O_2$	208.052	1094	0.063
(Phenanthrene or Anthracene) Quinone	$C_{14}H_{10}O_2$	208.052	1105	0.070
Dimethyl (Anthracene or Phenanthrene)	$C_{16}H_{14}$	206.109	1111	0.057
9, 10 Anthracene Quinone	$C_{14}H_{10}O_2$	208.052	1122	0.231
(Phenanthrene or Anthracene) Quinone	$C_{14}H_{10}O_2$	208.052	1142	0.044
Pyrene	$C_{16}H_{10}$	202.078	1157	0.237
Methyl 9, 10 Phenanthrene Quinone	$C_{15}H_{19}O_2$	222.068	1171	0.180
Unknown	$C_{15}H_8O$	204.057	1172	1.041
Trimethyl (Anthracene or Phenanthrene)	$C_{17}H_{16}$	220.125	1175	0.020
Methyl 9, 10 (Anthracene or Phenanthrene) Quinone	$C_{15}H_{10}O_2$	222.068	1193	0.092
Trimethyl (Anthracene or Phenanthrene)	$C_{17}H_{16}$	220.125	1199	0.095
Fluoranthene	$C_{16}H_{10}$	202.078	1205	0.190
Thioxanthone	$C_{13}H_8OS$	212.030	1208	0.057
Trimethyl (Anthracene or Phenanthrene)	$C_{17}H_{16}$	220.125	1217	0.046
Methyl 9, 10 Anthracene Quinone	$C_{15}H_{10}O_2$	222.068	1231	0.095
Pyrenone	$C_{16}H_{10}O$	218.073	1235	0.087
Thioxanthone	$C_{13}H_8OS$	212.030	1237	0.061
(Phenanthrene or Anthracene) Carboxaldehyde	$C_{15}H_{10}O$	206.073	1249	0.354
9-Thioxanthone	$C_{13}H_8OS$	212.030	1251	0.269
(Phenanthrene or Anthracene) Carboxaldehyde	$C_{15}H_{10}O$	206.073	1260	0.294
Fluoranthone or Pyrone	$C_{16}H_{10}O$	218.073	1270	0.041
Fluoranthone or Pyrone	$C_{16}H_{10}O$	218.073	1284	0.043
Methyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{16}H_{12}O$	220.089	1316	0.085
Unknown		226[3]	1318	0.024
Methyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{16}H_{12}O$	220.089	1338	0.188
Methyl Pyrene	$C_{17}H_{12}$	216.094	1338	0.024
Pyrene Quinone	$C_{16}H_8O_2$	232.052	1338	0.024
Methyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{16}H_{12}O$	220.089	1355	0.126
Methyl Fluoranthene	$C_{17}H_{12}$	216.094	1357	0.221

TABLE VI (Continued)

Compound	Formula	Mass[2]	Scan #	Relative abundance
Fluoranthene Quinone	$C_{16}H_8O_2$	232.052	1357	0.023
Methyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{16}H_{12}O$	220.089	1369	0.094
Dimethyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{17}H_{14}O$	234.104	1413	0.057
Pyrene or Fluoranthene Carboxaldehyde	$C_{17}H_{10}O$	230.073	1415	0.409
Dimethyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{17}H_{14}O$	234.104	1440	0.051
Benzo(d, h) Anthrone	$C_{17}H_{10}O$	230.073	1450	0.073
Dimethyl (Phenanthrene or Anthracene) Carboxaldehyde	$C_{17}H_{14}O$	234.104	1462	0.027
(Benzo(a) anthracene, Chrysene or Triphenylene) Carboxaldehyde	$C_{19}H_{12}O$	256.089	1499	0.032
(Benzo(a) anthracene, Chrysene or Triphenylene) Carboxaldehyde	$C_{19}H_{12}O$	256.089	1518	0.028
(Benzo(a) anthracene, Chrysene or Triphenylene) Carboxaldehyde	$C_{19}H_{12}O$	256.089	1523	0.028
(Benzo(a) anthracene, Chrysene or Triphenylene) Carboxaldehyde	$C_{19}H_{12}O$	256.089	1536	0.032
1-Nitro-Pyrene	$C_{16}H_9NO_2$	247.063	1624	0.027

[1]The relative abundance of 9-fluorenone is arbitrarily given a value of 1.000.
[2]Measured to within 11 ppm at 12K resolution.
[3]Mass accuracy is poor due to low ion abundance, therefore, exact empirical formula is difficult to establish.

TABLE VII

A comparison of the relative abundance of PAH species identified by direct probe HRMS and GC/MS for the transition (γ_1) HPLC fraction of a diesel particulate extract (OL-1)

Possible compounds	Formula	Mass[c]	Relative abundance		
			HRMS	GC/MS[b]	HRMS-GC/MS
9-Fluorenone	$C_{13}H_8O$	180.058	1.00	1.00	0.00
Hydroxy Fluorene†	$C_{13}H_{10}O$	182.073	0.15	0.00	0.15†
Naphthalene Dicarboxaldehyde	$C_{12}H_8O_2$	184.052	0.29	0.32	−0.03
Methyl (Anthracene or Phenanthrene)	$C_{15}H_{12}$	192.094	0.45	0.43	0.02
Anthrone or Phenanthrone	$C_{14}H_{10}O$	194.073	0.96	0.83	0.13†
Fluorene Carboxaldehyde					
Methyl Fluorenone					
Hydroxy (Anthracene or Phenanthrene)†					
Fluorene Quinone	$C_{13}H_8O_2$	196.052	0.66	0.04	0.62†
9-Xanthone					
Hydroxy Fluorenone†					
Hydroxy Xanthene†	$C_{13}H_{10}O_2$	198.068	0.30	0.00	0.30†
Dihydroxy Fluorene†					
Trimethyl Naphthalene Carboxaldehyde	$C_{14}H_{14}O$	198.104	0.29	0.19	0.10
Pyrene or Fluoranthene	$C_{17}H_{10}$	202.078	0.37	0.43	−0.05
Unknown	$C_{15}H_8O$	204.057	0.80	1.04	−0.24
(Anthracene or Phenanthrene) Carboxaldehyde	$C_{15}H_{10}O$	206.073	0.76	0.65	0.11
(Phenanthrene or Anthracene) Quinone	$C_{14}H_8O_2$	208.052	0.39	0.41	−0.02
Methyl (Anthrone or Phenanthrone)	$C_{15}H_{12}O$	208.089	0.35	0.39	−0.04
Thioxanthone	$C_{14}H_8OS$	212.030	0.47	0.39	0.08
Methyl Naphthalene Dicarboxylic Acid Anhydride	$C_{13}H_8O_3$	212.047	0.39	0.00	0.39†
Hydroxy Xanthrone					

TABLE VII (Continued)

Possible compounds	Formula	Mass	Relative abundance		
			HRMS	GC/MS	HRMS–GC/MS
Fluoranthone or Pyrone	$C_{16}H_{10}O$	218.073	<0.15	0.17	−0.02
Methyl (Anthrone or Phenanthrone) Dihydropyrone or Dihydrofluoranthone) Aceanthrone or Acephenanthrone	$C_{16}H_{12}O$	220.089	0.60	0.59	0.01
Methyl (Anthracene or Phenanthrene) Quinone Unknown	$C_{15}H_{10}O_2$	222.068	0.39	0.37	0.01
Dimethyl (Phenanthrone or Anthrone) Dimethyl Hydroxy (Phenanthrene or Anthracene)† Trimethyl Fluorenone	$C_{16}H_{14}O$	222.104	0.76	—	0.76†
Dihydroxy Methyl (Anthracene or Phenanthrene)†	$C_{15}H_{12}O_2$	224.084	0.44	—	0.44†
Hydroxy Nitro Fluorene†	$C_{13}H_9NO_3$	227.058	0.18	—	0.18†
(Pyrene or Fluoranthene) Carboxaldehyde Benzo (d, H) Anthrone	$C_{17}H_{10}O$	230.073	0.48	0.52	−0.04
Dimethyl (Anthracene or Phenanthrene) Carboxaldehyde Unknown†	$C_{17}H_{14}O$	234.104	0.49	0.14	0.35†
Dihydrodihydroxy (Pyrene or Fluoranthene)†	$C_{16}H_{12}O_2$	236.084	0.30	—	0.30†
Hydroxy Trimethyl (Anthracene or Phenanthrene)†	$C_{17}H_{16}O$	236.120	0.18	—	0.18†
Nitro Methyl (Anthracene or Phenanthrene)†	$C_{15}H_{11}NO_2$	237.079	0.14	—	0.14†

[a]†Indicates those species that were not eluted in the GC/MS procedure but may be present based upon HRMS relative abundance values.
[b]Errors in relative abundance values are +7%. GC/MS identifications for this fraction are presented in Table VI.
[c]Measured to within ±11 ppm at 12 K resolution.

TABLE VIII
Relative abundance of chemical species identified by GC/MS (DEXSIL 300) in the GC/MS analysis of a transition (γ_2) HPLC fraction of a diesel particulate extract (OL-1)

Compound	Formula	Mass	Scan #	Relative abundance
9-Fluorenone	$C_{13}H_8O$	180.058	719	0.157
Methyl Fluorenone	$C_{14}H_{10}O$	194.073	758	0.069
Methyl Fluorenone	$C_{14}H_{10}O$	194.073	787	0.041
Methyl Fluorenone	$C_{14}H_{10}O$	194.073	801	0.046
1 Naphto (c,d) Pyrone	$C_{12}H_8O_2$	184.052	808	0.105
Dimethyl Naphthalene Carboxaldehyde	$C_{13}H_{12}O$	184.089	846	0.040
Anthrone or Phenanthrone	$C_{14}H_{10}O$	194.073	853	0.175
Naphto, 2-3-B-Furan-4, 9-dione	$C_{12}H_6O_3$	198.032	862	0.099
Naphthalene Dicarboxaldehyde	$C_{12}H_8O_2$	184.052	863	0.054
Fluorene Quinone	$C_{13}H_8O_2$	196.052	876	0.033
Fluorene Quinone	$C_{13}H_8O_2$	196.052	888	0.040
9-Xanthone	$C_{13}H_8O_2$	196.052	900	0.073
Unknown	$C_{12}H_{10}O_3$	202.064	901	0.037
Fluorene Quinone	$C_{13}H_8O_2$	196.052	904	0.133
Unknown	$C_{12}H_{10}O_3$	202.064	912	0.030
Naphthalene Dicarboxylic Acid Anhydride	$C_{12}H_6O_3$	198.032	918	1.000
(Phenanthrene or Anthracene) Quinone	$C_{14}H_8O_2$	208.052	913	0.123
Methyl (Anthrone or Phenanthrone)	$C_{15}H_{12}O$	208.089	923	0.162
(Anthracene or Phenanthrene) Carboxaldehyde	$C_{15}H_{10}O$	206.073	928	0.020
Fluorene Quinone	$C_{13}H_8O_2$	196.052	935	0.053
(Phenanthrene or Anthracene) Quinone	$C_{14}H_{10}O_2$	208.052	948	0.064

TABLE VIII (Continued)

Compound	Formula	Mass	Scan #	Relative abundance
Methyl Naphthalene Dicarboxylic Acid Anhydride	$C_{13}H_8O_3$	212.047	970	0.123
Methyl Fluorene Quinone	$C_{14}H_{10}O_2$	210.068	973	0.102
Methyl Fluorene Quinone	$C_{14}H_{10}O_2$	210.068	979	0.091
Methyl Fluorene Quinone	$C_{14}H_{10}O_2$	210.068	995	0.005
Methyl Naphthalene Dicarboxylic Acid Anhydride	$C_{13}H_8O_3$	212.047	980	0.111
Dimethyl Fluorene Quinone	$C_{15}H_{12}O_2$	224.084	1010	0.043
Methyl Naphthalene Dicarboxylic Acid Anhydride	$C_{13}H_8O_3$	212.047	1015	0.108
Dimethyl Fluorene Quinone	$C_{15}H_{12}O_2$	224.084	1030	0.035
Dimethyl Naphthalene Dicarboxylic Acid Anhydride	$C_{14}H_{10}O_3$	226.064	1030	0.036
Dimethyl Fluorene Quinone	$C_{15}H_{12}O_2$	224.084	1055	0.021
Dimethyl Naphthalene Dicarboxylic Acid Anhydride	$C_{14}H_{10}O_3$	226.064	1059	0.120 (2 Isomers)
Dimethyl Naphthalene Dicarboxylic Acid Anhydride	$C_{14}H_{10}O_3$	226.064	1093	0.012
(Fluoranthene or Pyrene) Quinone	$C_{16}H_8O_2$	232.052	1148	0.026
Methyl (Pyrene or Fluoranthene) Quinone	$C_{17}H_{10}O_2$	246.068	1210	0.005
(Benzo(a) Anthracene, Chrysene or Triphenylene) Quinone	$C_{18}H_{10}O_2$	258.068	1225	0.006
(Benzo(a) Anthracene, Chrysene or Triphenylene) Quinone	$C_{18}H_{10}O_2$	258.068	1235	0.012

TABLE IX

A comparison of the relative abundance of PAH derivatives identified by direct probe HRMS and GC/MS for the transition (γ_2) HPLC fraction of a diesel particulate extract (OL-1)

Possible compounds	Formula	Mass	Relative abundance		
			HRMS[b]	GC/MS[c]	HRMS-GC/MS
9-Fluorenone	$C_{13}H_8O$	180.058	0.23	0.16	0.07
Hydroxy Fluorene†	$C_{13}H_{10}O$	182.072	0.30	—	0.30†
Naphthalene Dicarboxaldehyde	$C_{12}H_8O_2$	184.052	0.11	0.12	−0.01
Dimethyl Naphthalene Carboxaldehyde	$C_{13}H_{12}O$	184.089	0.11	0.03	0.08
Anthrone or Phenanthrone Methyl Fluorenone	$C_{14}H_{10}O$	194.073	0.30	0.33	−0.03
9-Xanthone; Fluorene Quinone	$C_{13}H_8O_2$	196.053	0.29	0.33	−0.04
Methyl Hydroxy Fluorene†	$C_{14}H_{12}O$	196.089	0.13	—	0.13†
Naphthalene Dicarboxylic Acid Anhydride	$C_{12}H_6O_3$	198.031	1.00	1.00	
Hydroxy Xanthene† Dihydroxy Fluorene†	$C_{13}H_8O_2$	198.067	0.38	—	0.38†
Unknown	$C_{12}H_{10}O_3$	202.064	0.05	0.07	−0.02
(Phenanthrene or Anthracene) Carboxaldehyde	$C_{15}H_{10}O$	206.073	0.03	0.02	0.01
(Phenanthrene or Anthracene) Quinone	$C_{14}H_8O_2$	208.052	0.20	0.19	0.01
Methyl (Anthrone or Phenanthrone) Dimethyl Fluorenone Methyl Hydroxy (Anthracene or Phenanthrene)†	$C_{15}H_{12}O$	208.089	0.23	0.15	0.07†
Methyl Fluorene Quinone; Dihydroxy Anthracene†	$C_{14}H_{10}O_2$	210.068	0.27	0.20	0.07†
Dimethyl Hydroxy Fluorene†	$C_{15}H_{14}O$	210.104	0.07	—	0.07†
Methyl Naphthalene Dicarboxylic Acid Anhydride	$C_{13}H_8O_3$	212.047	0.36	0.34	0.02

TABLE IX (Continued)

Possible compounds	Formula	Mass	Relative abundance		
			HRMS	GC/MS	HRMS–GC/MS
Dihydroxy Methyl Fluorene†	$C_{14}H_{12}O_2$	212.084	0.20	—	0.20†
Hydroxy Naphthalene Dicarboxylic Acid Anhydride†	$C_{12}H_6O_4$	214.026	0.32	—	0.32†
Trihydroxy Fluorene†	$C_{13}H_{10}O_3$	214.063	0.06	—	0.06†
Methyl (Anthracene or Phenanthrene) Quinone	$C_{15}H_{10}O_2$	222.068	0.26	(?)	—
(Anthracene or Phenanthrene) Dicarboxylic Acid Anhydride	$C_{14}H_8O_3$	224.047	0.21	[d]	—
Dimethyl Fluorene Quinone	$C_{15}H_{12}O_2$	224.084	0.17	0.10	0.07
Dimethyl Naphthalene Dicarboxylic Acid Anhydride	$C_{14}H_{10}O_3$	226.064	0.16	0.17	−0.01
Dihydroxy Methyl Fluorene†	$C_{15}H_{14}O_2$	226.099	0.08	—	0.08†
Unknown†	$C_{13}H_8O_4$	228.042	0.06	—	0.06†
Unknown†	$C_{14}H_{12}O_3$	228.078	0.03	—	0.06†
(Fluoranthene or Pyrene) Quinone	$C_{16}H_8O_2$	232.052	0.04	0.03	0.01
Dihydroxy Dimethyl Anthracene†	$C_{16}H_{14}O_2$	238.100	0.08	—	0.08†
Unknown†	$C_{15}H_{14}O_3$	242.094	0.03	—	0.03†

[a]†Indicates those species that were not eluted in the GC/MS procedure but may be present based upon HRMS relative abundance values.
[b]Measured to within ±11 ppm at 12K resolution.
[c]GC/MS identifications for this fraction are presented in Table VIII.
[d]Presence of compound not confirmed by GC/MS.

Hydroxy derivatives of PAH

A number of experiments were undertaken to understand the possible conversion of PAH and oxy-PAH during the analytical procedures. It was found that hydroxy, and dihydroxy derivatives of PAH were easily oxidized during analysis.

Conversion of some hydroxy-substituted PAH species during analysis is summarized in Table X. 9-Hydroxy fluorene was converted to the 9-fluorenone with 96 and 15% efficiency when analyzed by direct probe (glass) high resolution mass spectrometry at 200 and 115°C, respectively. The use of a gold probe greatly reduced the degree of this reaction (14% conversion at 240°C). Conversion of 4-OH benzo(a) pyrene to benzo(a) pyrene quinone (5–16%) was observed, depending upon conditions.

The 3-hydroxy benzo(a) pyrene was very reactive, exhibiting oxidation during storage of samples, and during HPLC and mass spectrometric analysis. Attempts to quantitatively elute the hydroxy-PAH species without derivatization were not successful. The 9-hydroxy fluorene was converted (62% efficiency) to the fluorenone during GC/MS analysis. Under these conditions, the fluorenone was eluted as a broad peak as would be expected for decomposition or oxidation of a species during analysis. These reactions may account for the low concentration of quinone-PAH found in the γ_1 fraction (see discussion on quinones).

The mass spectra for the ketone and hydroxy derivatives of the PAH were indistinguishable except for the case of 9-hydroxy fluorene and 9-fluorenone (Table XI). However, the hydroxy PAH were not eluted through the GC and therefore their possible presence may be inferred by comparing the HRMS and GC/MS results as described earlier.

There was a significant ion peak found at m/z: 196.052 ($C_{13}H_8O_2$) in the direct insertion probe HRMS analysis of the γ_1 fraction. Possible structures with this empirical formula include fluorene quinone, hydroxy fluorenone and 9-xanthone (Table VII). The only one of these species identified by GC/MS is 9-xanthone in scan #1004 (Table VI). Fluorene quinone was not identified in this fraction as would be expected since it is eluted in the γ_2 HPLC fraction (Table III). Therefore, the residual abundance of 0.62 could be accounted for by the presence of hydroxy fluorenone species. Hydroxy fluorenone was found to elute within the γ_1, HPLC fraction (Table III). Other hydroxy-PAH species that may be present incude those identified with a star in Table VII.

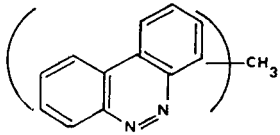
Ketone derivatives of PAH

A number of ketone derivatives of PAH were found in the γ_1 and γ_2 transition fractions (Tables VI-IX). The possible presence of this com-

pound class may be inferred by the time concurrent elution of abundant $(M)^{+\cdot}$, $(M-CO)^{+\cdot}$ and $(M-COH)^{+\cdot}$ ions in the mass chromatograms. Other ions originating from $(M-H)^{+\cdot}$, and $(M-COH_2)^{+\cdot}$ fragmentation processes are usually less than 15% the abundance of the molecular ion (Table XI).

Figure 6 shows the mass chromatograms used to identify three ketone isomers of anthracene and/or phenanthrene. The fragmentation patterns for these three isomers are quite similar and exact isomer identifications are difficult to make without comparison to elution times of standards. Only 9-anthrone could be positively identified (scan #947). Standards for the other isomers were not available.

The ketone derivatives of anthracene and phenanthrene could not be distinguished from the methyl benzo-(c)-cinnolines (I) by low resolution mass spectrometry since their fragmentation patterns are very similar.



I

However, methyl benzo-(c)-cinnolines ($C_{13}H_{10}N_2$-m/z: 194.0844) were not found to be present as determined using HRMS. The only ion found at nominal mass 194 has an exact mass of 194.0732 which is in good agreement with the exact mass of the anthrones and phenanthrones

TABLE X

Conversion of some hydroxy and ketone derivatives of PAH during various analytical procedures

Conditions	Reaction (percent conversion)		
	9-OH Fl to Fl ketone[a]	4-OH BaP to BaP Quinone[b]	3-OH BaP to BaP Quinone[b]
Direct probe HRMS			
200°C (glass probe)	96	16	>96
115°C (glass probe)	15	9	>96
240°C (gold probe)	14	5	—
GCMS			
Dexsil 400 column[c]	62(38)	—	—
HPLC			
Biosil normal			
Phase column	<2	<2	5–50[d]

[a]9-Hydroxy fluorene to fluorenone

[b]Hydroxy benzo(a) pyrene to benzo(a) pyrene quinone

[c]Based upon area of conversion product eluted from GC/MS system. Percentage in parentheses represents material unaccounted for.

[d]Range of conversions dependent upon age of column.

TABLE XI
Mass spectra for some nitrogen and oxygen containing derivatives of PAH

Compound	Mass (relative abundance)[a]				
	1	2	3	4	5
Hydroxy—PAH					
	$(M)^+$	$(M-CO)^+$	$(M-COH)^+$	$(M-COH_2)^+$	$(M-COH_3)^+$
2-Hydroxy-Naphthalene	142 (1.00)	114 (0.70)	113 (0.20)	—	—
9-Hydroxy Fluorene	182 (0.85)	154 (0.40)	153 (0.15)	152 (0.05)	151 (<0.05)
1-Hydroxy Phenanthrene	194 (1.00)	166 (0.15)	165 (0.80)	164 (0.10)	163 (0.05)
5-Hydroxy Benzo(a) Anthracene	244 (1.00)	216 (0.30)	215 (0.55)	214 (<0.05)	213 (0.15)
6-Hydroxy Benzo(a) Pyrene	268 (1.00)	240 (0.10)	239 (0.40)	238 (0.05)	237 (0.10)
7-Hydroxy Benzo(a) Pyrene	268 (1.00)	240 (0.10)	239 (0.50)	238 (0.05)	237 (0.10)
Methylhydroxy–PAH					
	$(M)^+$	$(M-H)^+$	$(M-O)^+$	$(M-HO)^+$	$(M-H_2O)^+$
5-Methylhydroxy Benzophenanthrene	258 (0.20)	257 (<0.05)	242 (1.00)	241 (0.40)	—
PAH Semiquinones					
	$(M)^+$	$(M-CO)^+$	$(M-COH)^+$	$(M-COH_2)^+$	$(M-COH_3)^+$
9-Fluorenone	180 (1.00)	152 (0.30)	151 (0.15)	—	—
9-Anthrone	194 (1.00)	166 (0.15)	165 (0.60)	164 (0.10)	163 (0.10)
9-Xanthone	196 (1.00)	168 (0.30)	139 (0.15)[b]	—	—
9-Thioxanthone	212 (1.00)	184 (0.70)	152 (0.20)[c]	139 (0.30)[d]	—
7-Benzo(d, e) Anthrone	230 (1.00)	202 (0.30)	201 (0.10)	200 (0.15)	101.5 (0.25)

Compound	Mass (relative abundance)[a]				
	1	2	3	4	5
PAH-Carboxaldehydes					
	$(M)^+$	$(M-H)^+$	$(M-CO)^+$	$(M-HCO)^+$	$(M-H_2CO)^+$
1-Naphthalene Carboxaldehyde	156 (1.00)	155 (0.80)	128 (0.75)	127 (0.95)	126 (0.15)
9-Fluorene Carboxaldehyde	194 (1.00)	193 (0.30)	166 (0.30)	165 (0.75)	164 (0.25)
9-Anthracene Carboxaldehyde	206 (1.00)	205 (0.50)	178 (0.75)	177 (0.40)	176 (0.40)
1-Pyrene Carboxaldehyde	230 (1.00)	229 (0.50)	202 (0.40)	201 (0.60)	200 (0.35)
Benzophenanthrene Carboxaldehyde	256 (1.00)	255 (0.45)	228 (0.65)	227 (0.30)	226 (0.50)
PAH-Dicarboxaldehydes					
	$(M)^+$	$(M-CO)^+$	$(M-HCO)^+$	$(M-HC_2O_2)^+$	$(M-H_2C_2O_2)^+$
Benzanthracene Dicarboxaldehyde	260 (0.50)	232 (0.25)	231 (1.00)	203 (0.20)	202 (0.45)
Dibenz (a, h) Anthracene Dicarboxaldehyde	310 (0.50)	282 (0.30)	281 (1.00)	253 (0.20)	252 (0.40)
Methyl PAH-Carboxaldehydes	$(M)^+$	$(M-H)^+$	$(M-CH_3)^+$	$(M-CO)^+$	$(M-HCO)^+$
2-Methyl-9-Anthracene Carboxaldehyde	220 (1.00)	219 (0.50)	205 (0.10)	192 (0.30)	191 (0.65)
PAH-Quinones					
	$(M)^+$	$(M-CO)^+$	$(M-2CO)^+$	$(M-HC_2O_2)^+$	$(M-H_2C_2O)^+$
1, 4 Naphthalene Quinone	158 (1.00)	130 (0.30)	102 (0.40)	—	—
1, 4, Fluorene Quinone	196 (0.85)	168 (1.00)	140 (0.60)	139 (0.10)	138 (0.05)
9, 10-Anthracene Quinone	208 (1.00)	180 (0.75)	152 (0.50)	151 (0.10)	150 (0.05)
3, 10-Pyrene Quinone	232 (1.00)	204 (0.25)	176 (0.50)	175 (0.10)	174 (0.10)
7, 12-Benzo (a) Anthracene Quinone	258 (1.00)	230 (0.30)	202 (0.30)	—	—
1, 6 Benzo (a) Pyrene Quinone	282 (1.00)	254 (0.30)	226 (0.30)	225 (0.10)	224 (0.15)
3, 6 Benzo (a) Pyrene Quinone	282 (1.00)	254 (0.25)	226 (0.25)	225 (0.10)	224 (0.15)
Methyl PAH-Quinones					
	$(M)^+$	$(M-CH_3)^+$	$(M-CO)^+$	$(M-C_3H_3O_2)^+$	$(M-C_3H_4O_2)^+$
2-Methyl-9, 10 Anthracene Quinone	222 (1.00)	207 (0.05)	194 (0.30)	166 (1.00)	165 (0.10)

Dihydroxy-PAH

	$(M)^+$	$(M-OH)^+$	$(M-H_2O)^+$	$(M-2OH)^+$	$(M-CO_2H_2)^+$
5, 6-Dihydrodiol Benzo(a) Anthracene	262 (1.00)	245 (0.25)	244 (0.50)	228 (0.25)	215 (0.95)
1, 6-Dihydrodiol Benzo(a) Pyrene	286 (0.20)	269 (0.30)	268 (1.00)	252 (0.10)	239 (0.40)
4, 5-Dihydrodiol Benzo(a) Pyrene	286 (1.00)	269 (0.45)	268 (0.80)	252 (0.10)	239 (0.50)

Nitro-PAH

	$(M)^+$	$(M-NO)^+$	$(M-NO_2)^+$	$(M-HNO_2)^+$	$(M-H_2NO_2)^+$
2-Nitro Fluorene	211 (0.75)	181 (0.10)	165 (1.00)	164 (0.35)	163 (0.30)
1-Nitro Pyrene	247 (1.00)	217 (0.40)	201 (1.00)	200 (0.55)	189 (0.40)
3-nitro-9-Fluorenone	225 (1.00)	195 (0.15)	179 (0.35)	—	—
6-Nitro Benzo(a) Pyrene	297 (0.80)	267 (1.00)	251 (0.65)	250 (0.65)	239 (0.50)

Dinitro-PAH

	$(M)^+$	$(M-NO)^+$	$(M-NO_2)^+$	$(M-2NO)^+$	$(M-2NO_2)^+$
1, 5-Dinitro Naphthalene	218 (1.00)	188 (0.10)	172 (0.05)	158 (0.02)	126 (0.95)
2, 7-Dinitro Fluorene	256 (1.00)	226 (0.15)	210 (0.45)	196 (0.05)	164 (0.65)
2, 7-Dinitro Fluorenone	270 (1.00)	254 (0.05)	224 (0.30)	210 (0.10)	178 (0.15)

PAH-Carboxylic Acid

	$(M)^+$	$(M-OH)^+$	$(M-CO_2H)^+$	—	—
1-Naphthalene Carboxylic Acid	172 (1.00)	155 (0.70)	127 (0.50)	—	—
2-Naphthalene Carboxylic Acid	172 (1,00)	155 (0.50)	127 (0.50)	—	—

PAH-Dicarboxylic Acid Anhydride

	$(M)^+$	$(M-CO_2)^+$	$(M-C_2O_3)^+$	—	—
1, 8-Naphthalene Dicarboxylic Acid Anhydride	198 (0.95)	154 (0.99)	126 (1.00)	—	—

[a]Abundance not listed if less than 0.05.
[b]$139(M-C_2O_2H)$
[c]$152(M-COS)$
[d]$139(M-C_2SOH)$

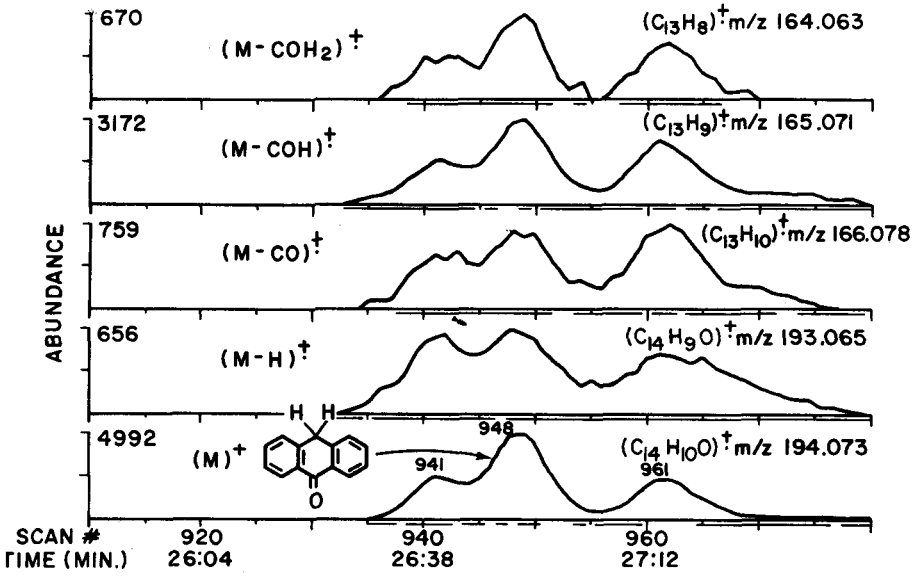
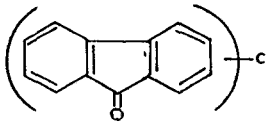


FIGURE 6 High accuracy mass chromatograms used for the identification of 9-anthrone in the γ_1 HPLC fraction of a diesel particulate extract OL-1 utilizing a 6′ SP2250 column (see Table IV).

($C_{14}H_{10}O$) at m/z: 194.0725. In addition, benzo-(c)-cinnoline elutes in the δ_1 region (Table III — 31.9 min) and not in the γ_1, or γ_2 regions. These findings are consistent with that of Erickson *et al.*,[38] who reported that benzo-(c)-cinnoline was not present as determined by FT-IR results.

9-fluorenone was the most abundant chemical species in the γ_1 fraction. It eluted at 21.0 min, in the γ_1 HPLC fraction (Table III). This is near the point at which the γ_1 and γ_2 fractions were split and explains why some of this species was found in the γ_2 fraction.

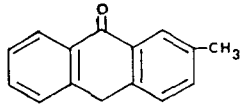
The methyl-9-fluorenones (II) give strong parent ions at m/z: 194.073, and major fragments at $(M-CO)^{+\cdot}$ and $(M-C_2H_3O)^{+\cdot}$ and a minor fragment at $(M-CH_3)^+$. The presence of the $(M-CH_3)^+$ ion was used to distinguish these species from the anthrones or phenanthrones.



II

Two methyl (anthrones or phenanthrones) were identified in scans #1057 and #1077 (Table VI). Figure 7 shows high accuracy ($\pm$30 ppm)

mass chromatograms (at 1500 resolution) for the major ions $(M)^+$, $(M-CH^3)^+$. The peak at scan #1057 was tenatively identified as 2-methyl-9-anthrone (III) as based upon the retention time of a known standard. However, even with this information, positive identification is difficult when the large number of possible isomers is considered.



III

The exact structure of the other isomeric species at scan #1077 is unknown.

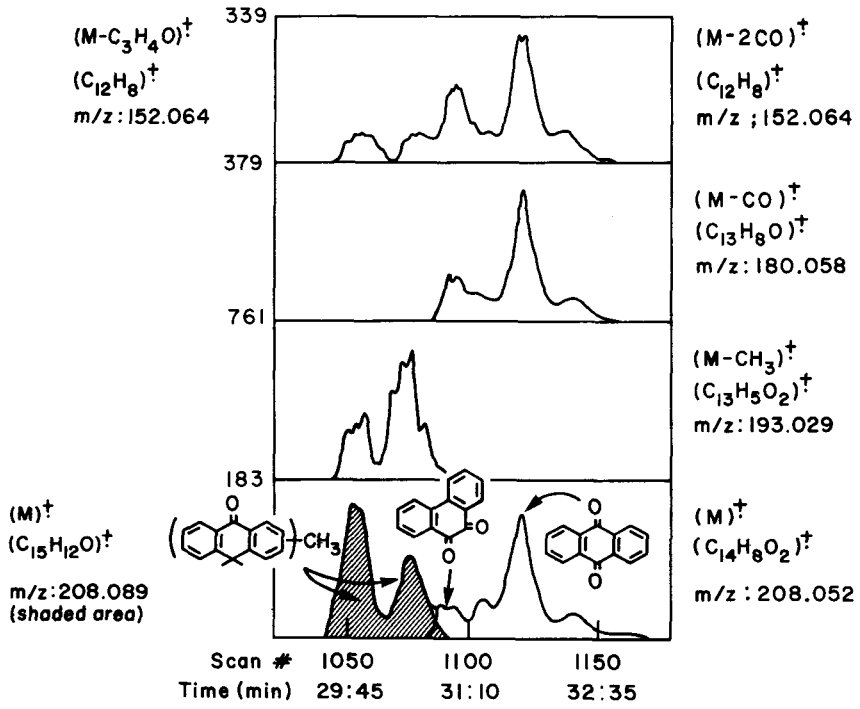
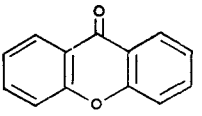


FIGURE 7 High accuracy mass chromatograms used for the identification of 9,10 phenanthrene quinone, 9.10 anthracene quinone and two methyl (anthrones or phenanthrones) in the γ_1 HPLC fraction of diesel particulate extract OL-1 utilizing a 6′ SP2250 column.

9-xanthone (IV) was identified in the γ_1 and γ_2 fractions (Table VI-scan #1004 and Table VIII-scan #900, respectively).



IV

9-xanthone gives a large parent mass at m/z: 196.0524 (196.0522 measured) and a major fragment of 30% relative abundance at m/z 168.058 (168.056 measured). This fragment represents loss of CO from the parent ion.

9-thioxanthone ($C_{13}H_8OS$-212.030) was identified in the γ_1 (Figure 4 scan #1521) fraction. 9-thioxanthone gives a large parent mass at m/z: 212 (relative abundance: 1.00) and major fragments at 184 (0.79) from loss of CO, 152 (0.20) from loss of COS and 139 (0.30) from loss of C_2SOH. The exact masses of these ions were found to be within 10 ppm as determined by HRMS. Two other isomers of thioxanthone were found at scans #1208 and #1237.

Carboxaldehyde derivatives of PAH

The carboxaldehyde derivatives of the PAH were among the most abundant PAH derivatives found in the transition fractions. These compounds were distinguished from other PAH-oxidation products by their abundant $(M-H)^+$ ions resulting from the loss of a hydrogen atom from the aldehyde group. The abundance of this ion showed little variation among isomeric species with a relative abundance of approximately 40–60% compared to the parent ion. Other significant ions include those originating from $(M-H)^{+\cdot}$, and $(M-COH)^{+\cdot}$ fragmentation processes (Table XI).

Figure 8 shows the mass chromatograms used to identify two isomers of anthracene or phenanthrene carboxaldehyde. It can be seen from the mass chromatograms that the relative abundances of the four major ions are nearly similar. Based upon the retention times of standards, these two isomers are identified as 9-phenanthrene and 9-anthracene carboxaldehyde. However, there is little difference between the retention times of standards making conclusive identification difficult.

Figure 9 shows the mass chromatograms used to identify the presence of several methyl (anthracene and phenanthrene) carboxaldehydes. The $(M-CH_3)^{+\cdot}$ ion is also present (not shown) at approximately 10% abundance of the $(M)^{+\cdot}$ ion. The relative abundances of all major ions from each isomeric species are nearly equivalent.

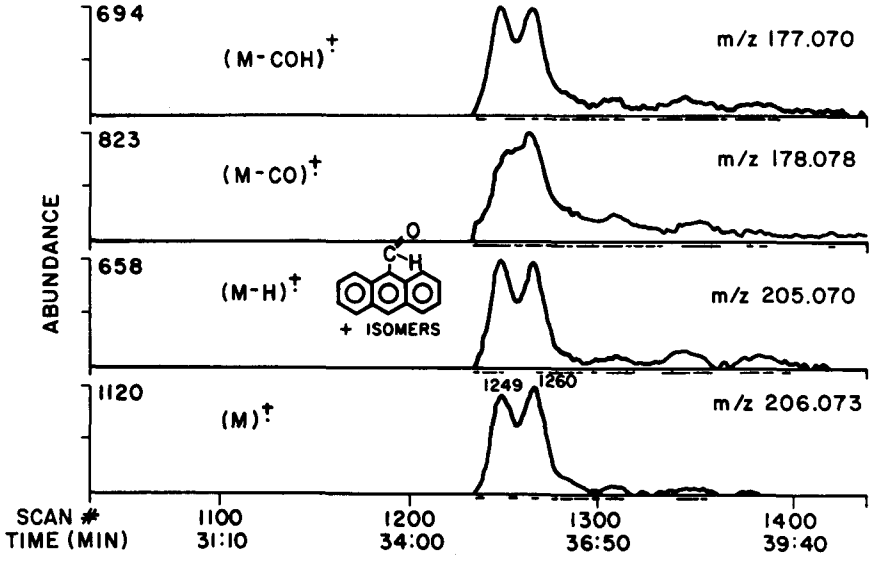


FIGURE 8 High accuracy mass chromatograms used for the identification of two (anthracene or phenanthrene) carboxaldehydes in the γ_1 HPLC fraction of a diesel particulate extract OL-1 utilizing a 6′ SP2250 column.

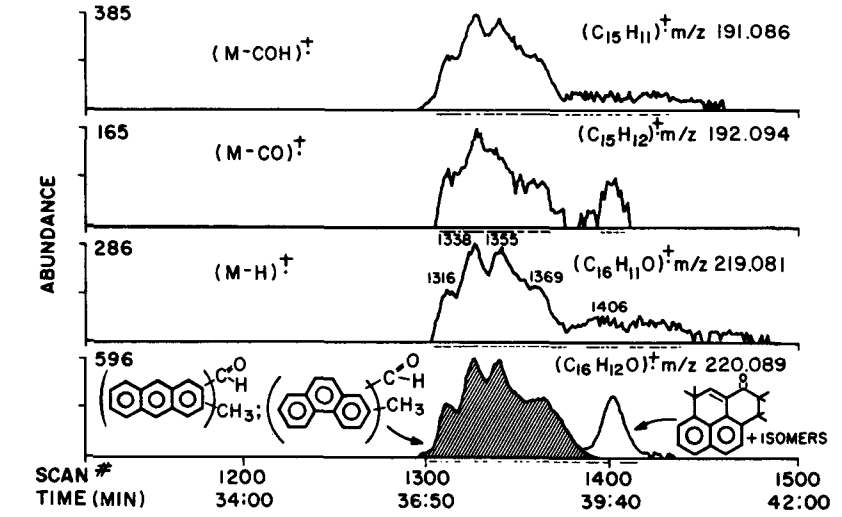


FIGURE 9 Mass chromatograms used for the identification of methyl (anthracene and phenanthrene) carboxaldehydes and a dihydropyrone in the γ_1 HPLC fraction of diesel particulate extract OL-1 utilizing a 6′ SP2250 column.

Quinone derivatives of PAH

The PAH-quinones are relatively easy to distinguish from the other PAH derivatives. The quinones all give strong molecular ion peaks as well as peaks corresponding to the loss of one and two molecules of carbon monoxide. Although the isomeric quinones yield the same molecular ion and fragment peaks, the relative heights of the peaks are quite different and therefore are used as an aid in identification. Figure 7 shows the high accuracy mass chromatograms used to identify 9, 10 anthracene quinone and 9, 10 phenanthrene quinone (m/z: 208.052) in fraction γ_1. The relative abundance of $(M-CO)^{+\cdot}$ to $(M)^{+\cdot}$ is 0.33 and 0.92 for 9, 10 anthracene quinone and 9, 10 phenanthrene quinones, respectively. Two isomers of methyl (anthrone or phenanthrone) were also present at nominal mass 208, and were easily distinguished from the quinones by the HRMS data and distinctive fragmentation patterns. Three methyl (anthracene and phenanthrene) quinones were identified in Fraction γ_1 (Table VI). Distinguishing features of the mass spectra are the presence of $(M)^{+\cdot}$, $(M-CH_3)^{+\cdot}$ and $(M-2CO)^{+\cdot}$ ions. The relative abundance of the $C_{15}H_{10}O_2^{+\cdot}$ (m z: 220.068) ion as given by HRMS and GC/MS (Table VII) agree within experimental error which indicates that all species with this atomic composition are accounted for.

The quinones of naphthalene were not detected in either fraction γ_1 or γ_2. A standard spectrum for the 1, 4 Naphthalenequinone isomer is given in Table XI.

Nitro derivatives of PAH

Some of the nitro-PAH species are difficult to analyze by GC/MS because of their high reactivity. Both nitro fluorene and nitro pyrene were found to elute from the SP2250 column described in this study. However, there appears to be some loss of these species during analysis.

Nitro naphthalene and nitro phenanthrene have been previously determined in solvent extracts of polymeric carbon by GC/MS using a 6 ft $\times$ 1/2 in packed column of Dexsil 300.[50,51] In this study, the analyses of nitro-PAH was undertaken through the use of HPLC fractionation followed by direct probe high resolution mass spectrometric (HRMS) and GC/MS analysis.

The electron impact (EI) mass spectra of nitro-PAH give simple spectra consisting of abundant $(M)^{+\cdot}$, $(M-NO)^{+\cdot}$ and $(M-NO_2)^{+\cdot}$ ions (see Table XI). Other important ions include those originating from '$M-HNO_2)^{+\cdot}$ and $(M-H_2NO_2)^{+\cdot}$ fragmentation processes.

A number of nitro-PAH standards were eluted through the high

resolution HPLC column (Figure 2) to determine their retention time (Table III). Elution times for the nitro-PAH were as follows: 2-nitro fluorene (17.0 min), 2-nitro naphthalene (17.5 min), 1-nitro pyrene (19.5 min), 6-nitro benzo(a) pyrene (20.5 min) and 2, 7 dinitro fluorene (22.4 min).

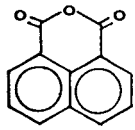
A fraction was isolated from the leading edge of the γ_1 peak (see Figure 2 — 17.0–20.0 min-γ_{1x}, fraction) and analyzed by HRMS at 12–15 K resolution. 1-nitro pyrene ($C_{16}H_9NO_2$) was positively identified in this fraction. The molecular mass of 1-nitropyrene was found experimentally to be 247.064 compared to the theoretical value of 247.063. The relative abundance of the $(M)^{+\cdot}/(M-NO_2)^{+\cdot}$ and $(M)^{+\cdot}/(M-HNO_2)^{+\cdot}$ ions were found to be 1.04 and 0.57, respectively which compares closely with the standard spectra (Table XI). This compound represents 0.9% of the total ions generated in the HRMS analysis for this fraction, and the total γ_{1x} fraction accounts for 1.6 wgt% of the extract mass. Therefore the 1-nitropyrene accounts for 144 ppm of the total extract.

1-nitropyrene was found also by GC/MS analysis of the γ_1 fraction at scan #1624 (Table VI). The relative abundances of the $(M)^{+\cdot}/(M\text{-}NO_2)^{+\cdot}$ and $(M)^{+\cdot}/(M\text{-}HNO_2)^{+\cdot}$ ions were found to be 1.02 and 0.71, respectively (see Table XI). It can be calculated that the 1-nitropyrene represents 0.32% of the total identified species. Approximately 60% of the ions generated from the GC/MS analysis were accounted for as described earlier. Another 40% of the ions (Table VII) not eluted through the GC/MS were eluted from the direct probe HRMS analysis. Therefore, the 1-nitropyrene is estimated to account for 0.19 wgt% of the γ_1 fraction mass and 131 ppm of the total extract mass (note that the conversion from ion abundance to wgt% assumes equivalent molecular weights and MS response factors for all identified species). The average 1-nitropyrene concentration from these two analyses is 137 ppm.

Nitro methyl (anthracene or phenanthrene) was tentatively identified in this fraction ($C_{15}H_{11}NO_2$-m/z: 237.079). The relative abundances of the $(M)^{+\cdot}/(M-NO_2)^{+\cdot}$ ions were found to be 0.83. Synthesis of these compounds would be required to establish the exact isomer structures.

Acid Anhydride derivatives of PAH

The most abundant component found in the γ-2 fraction was 1,8-naphthalene dicarboxylic acid anhydride (V)-(Table VIII-scan #918).



V

Major ions used for the MS identification include $(M)^{+\cdot}$, $(M-CO_2)^{+\cdot}$, and $(M-C_2O_3)^{+\cdot}$ as given in Table XI.

Three methyl naphthalene dicarboxylic acid anhydride isomers were also found in this fraction and identified by the presence of $(M)^{+\cdot}$, $(M-CH_3)^{+\cdot}$, $(M-C_2H_3O_2)^{+\cdot}$ and $(M-C_2O_3)^{+\cdot}$ ions (table VIII-scans #970, #980 and #1015).

Other acid anhydrides identified in this fraction included hydroxy naphthalene dicarboxylic acid anhydride, (anthracene or phenanthrene) dicarboxylic acid anhydride, dimethyl naphthalene dicarboxylic acid anhydride (tenative identification), and fluorene dicarboxylic acid anhydride (Tables VI-IX).

Acid derivatives of PAH

Naphthalic acid ($C_{11}H_8O_2$; m/z: 172.052), methyl naphthalic acid ($C_{12}H_{10}O_2$; m/z: 186.068) and dimethyl naphthalic acid ($C_{13}H_{12}O_2$; m/z: 200.084) were the most abundant components found in the δ fraction.

SUMMARY AND CONCLUSIONS

A variety of HPLC, GC/MS and high resolution mass spectrometric techniques have been used to determine the composition of several fractions obtained by the HPLC separation of diesel particulate extracts. These fractions have been designated as non-polar (PAH: α_1, α_2, β), moderately polar (transition: γ_1, γ_2) and polar subfractions (oxygenates: δ_1 and δ_2).

The mass recovery of the diesel extract injected into the HPLC was greater than 96% utilizing the low-resolution normal phase column and a four step solvent elution procedure.

It was found that 103% of the direct acting mutagenicity and 91% of the indirect acting mutagenicity injected into the HPLC was recovered. Two important conclusions may be drawn from this data: 1). the mutagenicity of the total extract was equivalent to the summation of its fractions and 2). the normal phase silica column did not significantly modify the mutagenicity of the extract.

Short-term bioassays were found to be extremely useful for defining the major fractions which should be prioritized for chemical analysis. The γ_1 and γ_2 fractions were found to comprise more than 65% of the direct acting mutagenicity for the total extract and thus their chemical composition was studied in detail. The β fraction for sample NI-1 was chosen for detailed chemical analysis because of its relatively high HPLC

fluorescence and indirect acting Ames mutagenicity compared to that observed for a number of other diesel extracts.

α_1, α_2 *Fraction*—These fractions exhibited no detectable direct acting Ames mutagenic activity, whereas the relative mutagenicity of this fraction was increased to 16–20% by using S9 activation. This fraction represented the highest percentage of the total extract mass (53–57 wgt%) for the seven HPLC fractions described in this study. Most of the mass (85–90 wgt%) was due to the presence of aliphatic, and alkyl (benzenes and naphthalenes). PAH were identified in the MW range from 178 to 302 and methylated PAH were the most abundant PAH species.

A survey of the literature to date (Table XII) indicates that the PAH require enzymatic activation for mutagenicity response whereas some nitrated and oxygenated PAH have been shown to be direct acting mutagens. Nitrated and oxygenated PAH species were not detected in this fraction which is consistent with its observed lack of direct acting mutagenicity.

β *Fraction*—The β fraction did not show a significant level of direct acting mutagenicity when carefully separated by HPLC from the γ_1 fraction. Nitro fluorene and nitro naphthalene standards were found to elute in the region between the β and γ_1 HPLC peaks and therefore, care must be taken to insure that it is known in which fraction (β or γ_1) the nitro species are being collected. Order of magnitude variations in the direct acting mutagenicity were probably caused by varying amounts of nitro-PAH that were cut into the β fraction.

The β fraction contained species such as PAH, and bridged and methylated PAH. However, most of the lower molecular weight PAH species were present in this fraction because of incomplete separation from the α_1 and α_2 peaks (the β fraction begins to elute before the α_2 peak has reached a baseline level).

Varying quantities of oxygenated PAH(oxy-PAH) were found in each fraction, depending upon the parent PAH species present and the degree to which these species oxidize during storage and biological and chemical assays. For this reason, great care must be taken to minimize chemical conversion.

γ_1 *Fraction*—The γ_1 fraction showed the largest portion of direct and indirect acting mutagenicity. Sixty mole% of this fraction was composed of oxygenated PAH species. The remainder of this fraction consisted of aliphatic hydrocarbons and phthalate ester contaminants. These contaminants were difficult to eliminate because of their ubiquitous nature.

Approximately eighty PAH derivatives have been identified in this fraction. The most abundant oxy-PAH species (approximately 39 mole %

of the total identified species) were ketone and hydroxy-PAH derivatives containing 3–4 rings. 9-fluorenone was the most abundant species but does not show mutagenic activity for any of the TA98, TA1538, TA100 and TA1538 Salmonella typhimurium strains with or without activation.[58]

The presence of several hydroxy and dihydroxy-PAH species were inferred from a comparison of HRMS and GC/MS relative abundance data. Salmonella typhimurium mutagenicity data for these species are not available in the literature, except for hydroxy phenanthrene (Table XII).

It was difficult to determine accurately the concentration of the hydroxy and PAH-ketone species since there was interconversion and both species were found to undergo further oxidation during analysis, depending upon the conditions and species involved.

The carboxaldehyde PAH derivatives were the third most abundant species (22%) and represented substitutions to 2–5 rings. The only bioassay data reported for carboxaldehyde derivatives of PAH is for 6-BaP carboxaldehyde which has been found to induce high levels of tumor activity in mice.[58]

Minor components included PAH-quinones (6 mole %) containing 3–4 rings. Analytical data suggest that the higher MW quinones in this fraction arise from the oxidation of PAH or oxy-PAH (i.e. alcohols or ketones) during the analytical procedure. Several quinone derivatives have been found to be toxic to Salmonella typhimurium.[55] The presence of these quinones may partly explain why the dose response curves used for determination of mutagenic activity rapidly falls off with increasing sample concentration.[8]

Nitro methyl (anthracene or phenanthrene), nitro hydroxy fluorene and nitro dihydropyrene have been tentatively identified in this fraction pending synthesis of standards. 1-nitro pyrene has been positively identified in an early eluting subfraction (γ_{1x}) of the γ_1 fraction. The nitro-PAH were present as minor components (<0.5 mole %).

Nitro-PAH standards were found to elute in the γ_1 fraction. These nitro-PAH compounds may represent a significant portion of the fraction mutagenicity assuming their activity is similar to that of other nitro-PAH compounds which have been described in the literature (Table XII). Quantitative measurements of nitro-PAH will be required in order to assess their contribution to the total mutagenicity of this fraction.

Two dihydroxy dihydro-PAH derivatives of anthracene or phenanthrene and pyrene or fluoranthene (2 mole % abundance) have been tentatively identified. The mutagenicity of these species is unknown.

Oxygenated derivatives of heterocyclic PAH were identified in the γ_1 fraction as 9-thioxanthone, a thioxanthone isomer and 9-xanthone. The concentration of these species is minor representing approximately 3 mole % of the total species identified in the γ_1 fraction.

γ_2 *Fraction*—The γ-2 fraction showed direct acting mutagenicity

representing 9–15 % of the total extract activity and 2.3–5.0 % of the SOF mass for samples OL-1 and NI-1, respectively. This fraction was mostly comprised of oxygenated PAH species. The most abundant species were anhydrides of naphthalic and methyl naphthalic acid which represented the most abundant species (30 mole %). A number of other acid anhydrides were also found in this fraction. The ketone and quinone derivatives comprised 16 and 17 mole % of the

TABLE XII

Ames salmonella typhimurium mutagenicity for some PAH and nitrogen and oxygen containing derivatives of PAH

Compound	Activity (revertants/mg) TA98 −S9	TA98 +S9
N and O-PAH		
Benzo(e)pyrene-9, 10 Epoxide	—	2,700,000 (52)
1-Nitropyrene	1,400,000 (60)	—
Nitrobenzo(a)pyrene (1 + 3 Isomers)	707,000 (59)	17,500,000 (59)
4, 5-Dihydrodihydroxy Benzo(e)pyrene	—	480,000 (52)
2, 4, 7-Trinitrofluorenone	95,000 (57)	—
2, 7-Dinitrofluorenone	75,000 (57)	—
Benzo(e) pyrene-4, 5 Dihydrodiol	—	53,600 (52)
2, 7-Dinitrofluorene	35,000 (57)	—
2-Nitrofluorene	34,000 (23)	—
Acetylamino Fluorene	17,500 (57)	—
Pyrene Oxidation Products	8,400 (59)	—
Hydroxy Phenanthrene	7,100[a] (56)	—
9, 10 Phenanthrene Quinone	7,100[a] (56)	—
Benzo(a) Pyrene Oxidation Products	7,200 (59)	—
1, 5-Dinitro-2 Methyl 9, 10 Antraquinone	280[a] (47)	5,200[a](47)
Trihydroxy Anthraquinone (Anthralin)	1,700 (57)	—
Cyclopenteno (c, d) pyrene Anhydride	300 (26, 40)	—
Benzanthrone Oxidation Products	219 (59)	—
9, 10-Dihydroxy Anthracene	Toxic (56)[b]	Toxic (56)[b]
9, 10-Anthracene Quinone	Toxic (56)[b]	Toxic (56)[b]
PAH		
9, 10-Dihydro Benzo(e)pyrene	—	278,000 (52)
1, 9-Dimethyl Fluorene	—	80,000[a] (54)
Benzo(a)pyrene	—	45,000 (54)
10-Methyl Benzo(a)pyrene	—	33,000 (54)
Benz(a) Anthracene Fraction-Syncrude	—	6,600 (53)
PAH + ME − PAH Fraction-Syncrude	—	3,800 (53)
9-Methyl Fluorene	—	2,500[a] (54)
Multialkylated PAH Fraction-Syncrude	—	2,220 (53)

[a]TA100 Used instead of TA98.

PAH species eluted through the GC, respectively. Hydroxy-PAH were determined by HRMS and found to represent 25% of the identified species. Minor components included carboxaldehyde (1.2%), dicarboxaldehyde (1.1%), and dihydroxy (7.0%) derivatives of PAH.

The Ames mutagenicity for cyclopento (c, d) pyrene anhydride has been reported in the literature to be 300 revertants/mg (TA98i-S9). However, there are no mutagenicity data available for the other compounds identified in this fraction (Table XII).

δ_1 Fraction—The δ_1 fraction was primarily comprised of (PAH and alkyl substituted PAH) carboxylic and dicarboxylic acids. Less abundant components included heterocyclic-PAH and oxygenated derivatives of heterocyclic-PAH.

Origin of PAH Derivatives—Figure 10 shows the relative abundance of anthracene, phenanthrene and methylated derivatives of these PAH in the α_1, α_2 fraction. The oxy-(anthracene or phenanthrene) derivatives may be formed from the oxidation of parent or methyl substituted anthracene or phenanthrene to form quinone, hydroxy, ketone and dihydroxy derivatives while the oxidation of the methyl group could in addition form (anthracene or phenanthrene) carboxaldehydes and methanol derivatives. Carboxylic acid derivatives of PAH were found in the δ_1 fraction. Naphthalene dicarboxylic acids and anhydrides may be formed from oxidation and ring opening of phenanthrene and anthracene.[62] Figure 11

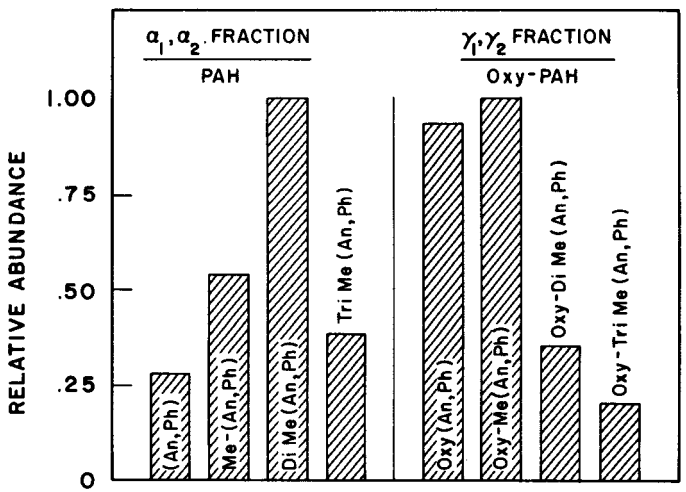


FIGURE 10 Relative abundance of anthracene (An) and phenanthrene (Ph) and methylated derivatives of anthracene (Me–An) and phenanthrene (Me–Ph) identified in the α_1, α_2 fraction and their oxidation products identified in the γ_1 and γ_2 fractions.

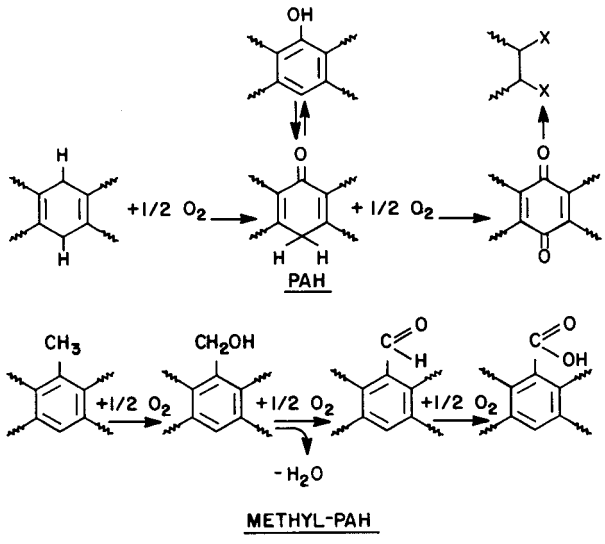


FIGURE 11 General scheme and possible PAH derivatives formed from the oxidation of PAH and methyl-PAH. × represents substituents such as COOH, CHO.

illustrates a general oxidation scheme postulated for PAH and methyl-PAH and the possible OXY-PAH products that may be formed.

It is unknown whether the oxy-PAH derivatives observed in this study were formed during the engine combustion process, in the exhaust system, in the sample collection process. Previous work in this laboratory has shown that PAH may be degraded on filter media.[32,41] Korfmacher *et al.*[62] found that PAH containing benzylic carbon atoms such as fluorene, benzo(a)fluorene, benzo(a)fluorene, 9, 10 dimethylanthracene and 9, 10 dihydroanthracene undergo rapid decomposition in the absence of light when adsorbed on fly ash or activated charcoal. Work was not undertaken in that study to determine the products of these degradations. However, it was found that nonphotochemical degradation of fluorene yielded 9-fluorenone.

It was found in this study that the oxy-PAH may be further oxidized to other oxy-PAH species during the analytical procedure. Chemical conversion of PAH species has been found during storage, extraction and liquid chromatographic separation and may significantly affect the results of subsequent chemical or biological studies. Further work in this area is needed.

Mutagenicity of PAH derivatives

Mutagenicity data for most of the PAH species identified in this study are not available. However, as described earlier, 1-nitropyrene was estimated to comprise 0.19 wgt% of the γ_1 fraction. Based upon this abundance, it is estimated that 1-nitropyrene accounts for 44% of the γ_1 fraction mutagenicity (2,660 revertants/mg) as determined from the activity of 1-nitropyrene (1.4×10^6 revertants/mg-Table XII) and the activity of the γ_1 fraction (OL-1 = 6100 revertants/mg). The concentration of 1-nitropyrene in the total extract (OL-1) was found to average 137 ppm as described earlier and therefore represents approximately 30% of the total extract mutagenicity. In addition, hydroxy nitro fluorene and nitro methyl(anthracene or phenanthrene) were tentively identified (Table VII). The mutagenicity of these species is not known. However, it is apparent that the nitro-PAH may account for a significant portion of the direct acting mutagenicity for this fraction. Better bioassay and chemical quantitation for the nitro-PAH will be required and because of their apparent importance will be the emphasis for future studies.

Since each class of PAH derivatives contain a large number of possible isomers, it would be nearly impossible to study the mutagenicity of every possible compound present in these fractions. Instead, future efforts should include acquisition of mutagenicity data for the general classes of PAH derivatives identified in this study. Further work could then be directed toward specific isomers if a particular class of PAH derivatives show high mutagenic activity.

It was difficult in these studies to obtain enough sample from each HPLC fraction for subsequent mutagenicity assay without loss of the analytical resolution required for well-defined fraction separation. However, additional mutagenicity data will be needed for sub-fractions of the seven major fractions in order to better understand the relationship between chemical composition and mutagenicity. Larger scale HPLC separations or more sensitive mutagenic assays are needed to carry on these studies.

Acknowledgement

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