Journal of Chromatography, 351 (1986) 451–464
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 210

FRACTIONATION OF POLAR POLYNUCLEAR AROMATIC HYDROCARBONS PRESENT IN INDUSTRIAL EMISSIONS AND ATMOSPHERIC SAMPLES AND THEIR DETERMINATION BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY*

P. CICCIOLI*, E. BRANCALEONI, A. CECINATO, C. DI PALO, P. BUTTINI and A. LIBERTI
Istituto Inquinamento Atmosferico del CNR, Area della Ricerca di Roma, Via Salaria Km 29,300, CP 10, 00016 Monterotondo Stazione, Rome (Italy)
(Received September 21st, 1985)

SUMMARY

A method for the fractionation of polar polynuclear aromatic hydrocarbons (PAHs) in industrial emissions and atmospheric samples is described, based on the sequential use of low- and high-resolution liquid chromatography for separating the various components according to their polarity. The fractions collected from the high-performance liquid chromatographic column are suitable for gas chromatographic and gas chromatographic–mass spectrometric analysis. The method has allowed the identification of several polar PAHs in real samples, such as diesel exhaust, industrial emissions and atmospheric dust. The fractionation scheme adopted should permit a correlation between the chemical composition of the slightly polar fraction and the direct mutagenic activity measured by microbiological assays.

INTRODUCTION

In the past 20 years, great attention has been devoted to the determination of polynuclear aromatic hydrocarbons (PAHs) in environmental samples, as these compounds are believed to be the major cause of the mutagenic activity as measured by the Ames test carried out in the presence of some activating agents[1]. The recent discovery[2,3] that the soluble organic fraction (SOF) extracted from certain anthropogenic sources (such as diesel exhaust and some industrial processes) can give a positive response to the Ames assay, when no metabolizing liver preparations are added to the *in vitro* bacterial system, has suggested that other genotoxic compounds capable of acting as direct mutagens, may be present in these samples. The results of chemical[4–8] and biological[9] analyses carried out on the SOF extracted from diesel particulates have clearly shown that directly acting mutagenicity is associated with

* Presented as a poster at the *A. J. P. Martin Honorary Symposium, Urbino, 27–31 May, 1985*. The majority of the research papers presented at this symposium has been published in *J. Chromatogr.*, Vol. 349, No. 1.

0021-9673/86/$03.50

the presence in the sample of polyaromatic compounds containing heteroatoms or polar functional groups in the molecule (PAH derivatives).

Although the presence of some PAH derivatives in particulate matter has been attributed to artifactual problems occurring during sampling[10], the occurrence of such artifacts has not yet been positively demonstrated and the general concern about possible health effects caused by PAH derivatives, particularly those containing nitro, hydroxy and carbonyl groups, has led to a need for specific determinations of these components in environmental samples.

Owing to the extreme dilution of PAH derivatives in the SOF, an enrichment process is essential for obtaining samples suitable for gas chromatographic (GC) and gas chromatographic–mass spectrometric (GC–MS) analysis. However, a fractionation procedure for PAH derivatives is not easy to design because of the great complexity of the SOF, the small differences in polarity and molecular size between PAHs and their derivatives and the large number of polyaromatic compounds produced during industrial processes and other human activities. Moreover, the possibility of collecting fractions containing the smallest number of individual mutagens also has to be taken into account if the chemical composition of the SOF is to be fully elucidated. Usually, the fractionation methods described in the literature have been designed for specific samples and difficulties are often experienced in adapting the same fractionation procedure to different matrices.

In this work we describe a procedure that can be used for separating PAH derivatives present in different samples. It consists in the sequential use of low- and high-resolution chromatography for fractionating the SOF. The various fractions and subfractions collected during the enrichment process are suitable for GC and GC–MS analysis. The procedure has been applied to the determination of PAH derivatives present in dust samples collected in the atmosphere of a suburban area, in the stack of an industrial plant and from the exhaust pipe of a light-duty diesel engine. Several different PAHs and PAH derivatives have been identified in these samples.

EXPERIMENTAL

Sampling procedure

Particulate matter emitted from a commercial automobile (1 year old), equipped with a 1.6 l diesel engine, was collected on a pre-cleaned glass-fibre filter 47 mm in diameter (Gelman, Spectroglass type), connected to a Gelman 4000 sampling apparatus, capable of maintaining a constant flow-rate during sampling. The sampling device was connected to the exhaust pipe through an expansion chamber, 10 l in volume, with the aim of preventing turbulent motion of the particles.

Particulate matter emitted from the stack of an industrial plant producing carbon electrodes was sampled isokinetically using quartz wool to retain the particles. The aspirating pump and the flow-control devices were the same as those used for the sampling of diesel particulates. The type of plant and the sampling site were selected specifically because substantial amounts of PAHs and PAH derivatives could be emitted when a mixture of coke powder and coal bitumen was passed, after extrusion, over a furnace kept at 800°C. As methane was used as the fuel for the oven, the dust collected from the stack was basically that emitted from coke and coal bitumen during the heating process.

Atmospheric dust sampled in a suburban area located 30 km downwind from Rome was collected on a 20 × 25 cm glass-fibre filter (Gelman, Spectroglass type) by using a high-volume Staplex pump. The pump and the filter holder were assembled in a sampler shelter constructed according to the recommendation of the International Agency for Research on Cancer[11].

Extraction procedure

All samples were extracted for 6 h in a Soxhlet apparatus (60 ml) by using 50 ml of benzene–methanol (4:1) as extraction mixture. The same mixture was used to clean the filters and the glass-wool prior to sampling. The extraction temperature was always kept below 80°C. After extraction, the SOF was evaporated to dryness at room temperature in the dark under a flow of ultrapure nitrogen. Prior to the LC fractionation, the residue was dissolved in a 10 ml of dichloromethane–methanol (2:1) mixture and passed through a glass filter (3 cm) covered with 2 g of basic alumina in order to eliminate carbon particles and polymeric residues, which could damage the chromatographic columns.

Low-resolution liquid chromatography

After filtration, the SOF was dried, dissolved in 200 μl of solvent (CH_2Cl_2–CH_3OH, 3:1) and an aliquot was injected into a semi-preparative column (320 × 8 mm I.D.) filled with basic alumina (70–150 mesh) (Woelm) (Carlo Erba, Milan, Italy). The degree of activation required for the separation of the SOF was obtained by heating the column to 250°C under a flow of ultrapure helium for at least 12 h. The SOF was injected into the column by using a Rheodyne 7105 injection valve (Rheodyne, Berkeley, CA, U.S.A.). Elution of the various fractions was performed with *n*-hexane, dichloromethane, acetonitrile and acetonitrile–methanol (3:1), used isocratically in that order. A Clar 003 (Violet, Rome, Italy) liquid chromatograph, equipped with a three-way valve for solvent delivery, was used for the separation.

The various fractions were detected by a UV absorption detector (Violet Clar 004) set at 254 nm. Four fractions, containing compounds of different polarity, were collected at the detector outlet. The first two fractions, containing aliphatic hydrocarbons and PAHs, respectively, were evaporated to dryness, dissolved in 100 μl of solvent and aliquots analysed directly by GC and GC–MS. The slightly polar fraction eluted with acetonitrile, which contains most of PAH derivatives, was evaporated to dryness, dissolved in 40 μl of dichloromethane, and further fractionated by HPLC. The polar fraction, eluted with acetonitrile–methanol (3:1), was analysed directly by GC–MS.

High-resolution liquid chromatography

The separation of the fraction containing most PAH derivatives, which are believed to act as direct mutagens, was carried out on 250 × 4 mm I.D. stainless-steel column, packed with 10-μm Erbasil silica (Carlo Erba). In order to obtain reproducible retention, the column was pre-cleaned with dichloromethane (20 min at a flow-rate of 1 ml/min) and then conditioned with *n*-hexane (20 min at a flow-rate of 1.5 ml/min) prior to every analysis. To check the column retention and sample recovery, a known amount of perdeuteronitronaphthalene was added to the fraction to be analysed. The separation was carried out by gradient elution using *n*-hexane

with the addition of dichloromethane at a rate of 2%/min. The number of subfractions to be collected for the GC and GC–MS analyses was decided on a case-by-case basis according to the complexity of the sample. After collection, these subfractions were dried and redissolved in dichloromethane and aliquots were taken for identification and quantification of PAH derivatives by GC and GC–MS.

GC and GC–MS

The GC separations were carried out on a Carlo Erba HRGC 5160 gas chromatograph, equipped with an on-column injector and a flame ionization detector, and on a Dani Model 6800 (Dani, Monza, Italy) equipped with a temperature-programmed vaporizer (TPV) injector and a Carlo Erba Model 40 nitrogen–phosphorus detector.

The GC–MS analyses were performed on a Dani 3900 gas chromatograph, connected directly to a VG 70-70 F double-focusing mass spectrometer (VG Analytical, Altrincham, U.K.) equipped with a VG 2350 Data System.

All GC separations were carried out on fused-silica capillary columns coated with DB-5 (J & W Scientific, Rancho, Cordova, CA, U.S.A.). The I.D. was 0.32 mm in each instance, whereas the column length and film thickness were selected according to the resolution required for the type of sample being analysed (either 30 m and 0.25 μm or 20 m and 0.52 μm).

RESULTS AND DISCUSSION

Fractionation of the SOF

Fig. 1a, b and c show the chromatographic profiles obtained when the SOF of the diesel exhaust, industrial emission, and suburban particulate matter, respectively, were fractionated on the semi-preparative alumina column. A comparison between the peaks corresponding to the elution of alkanes, PAHs, PAH derivatives and polar compounds gives an idea of the wide difference in composition between these three samples. As previous experiments, carried out by injecting standard solutions on the same alumina column, have shown that 1-nitropyrene, 1,8-dinitropyrene, carbazole, quinoline and benz[*a*]anthracen-7,12-dione were eluted in the acetonitrile fraction, whereas PAHs from naphthalene to benzo[*a*]pyrene were recovered in the dichloromethane eluate, an efficient separation between protomutagens (PAHs) and the most important possible direct mutagens (*e.g.*, nitro-PAHs, azaarenes and quinones) is obtained by submitting the SOF to this analytical step. The pre-fractionation on alumina is useful for two reasons: it gives a preliminary indication of the total content of polyaromatic compounds in the SOF and permits a rough estimate of the relative importance of protomutagens and directly acting mutagens in determing the biological activity of a given sample.

Analysis of the LC fractions

Fig. 2a, b and c show the chromatographic profiles of alkanes present in the same samples and Fig. 3a, b and c show the profiles for the PAH fractions. Table I lists those PAH derivatives (basically sulphur- and oxygen-containing compounds) that were found in the dichloromethane fraction. Whereas Fig. 2 exemplifies well the difference between natural (high-carbon preference index) and anthropogenic sources

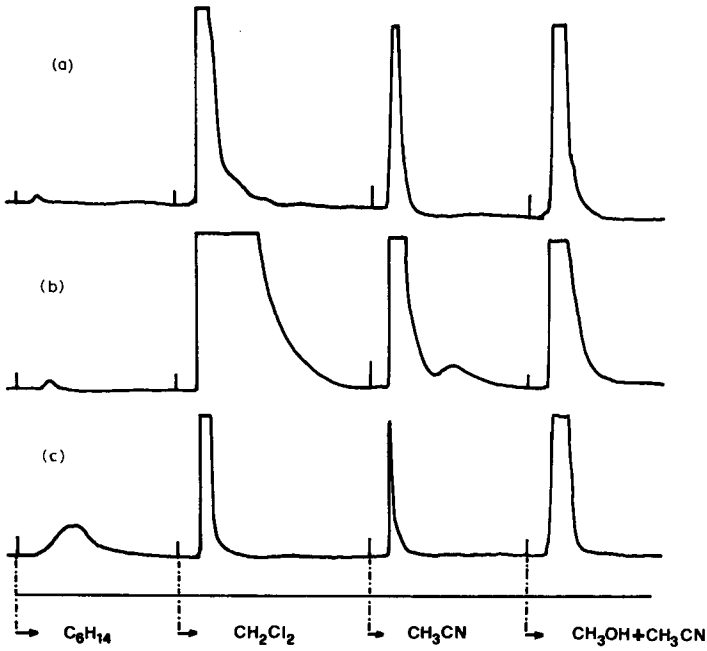

Fig. 1. Low-resolution LC of the SOF of different samples carried out on a semi-preparative column packed with basic alumina activated at 250°C under a flow of helium. The profiles relate to the particulate matter collected from (a) a diesel exhaust, (b) an industrial emission and (c) the atmosphere of a suburban area.

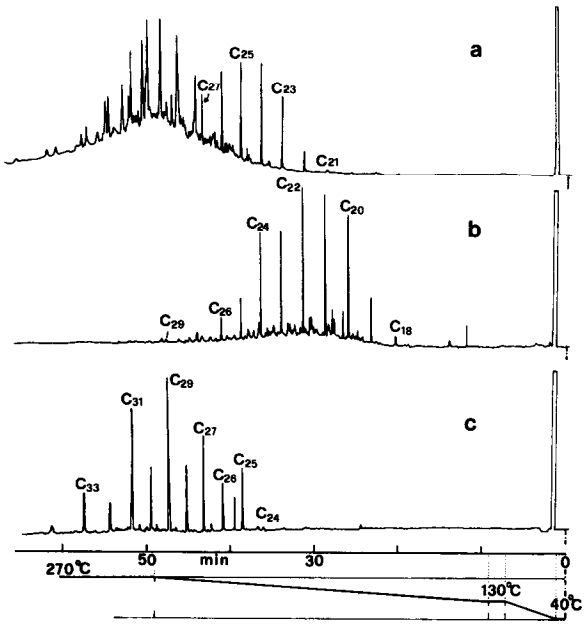

Fig. 2. GC separation of alkanes eluted from the alumina column (C_6H_{14} fraction). The letters a–c refer to the same samples as in Fig. 1.

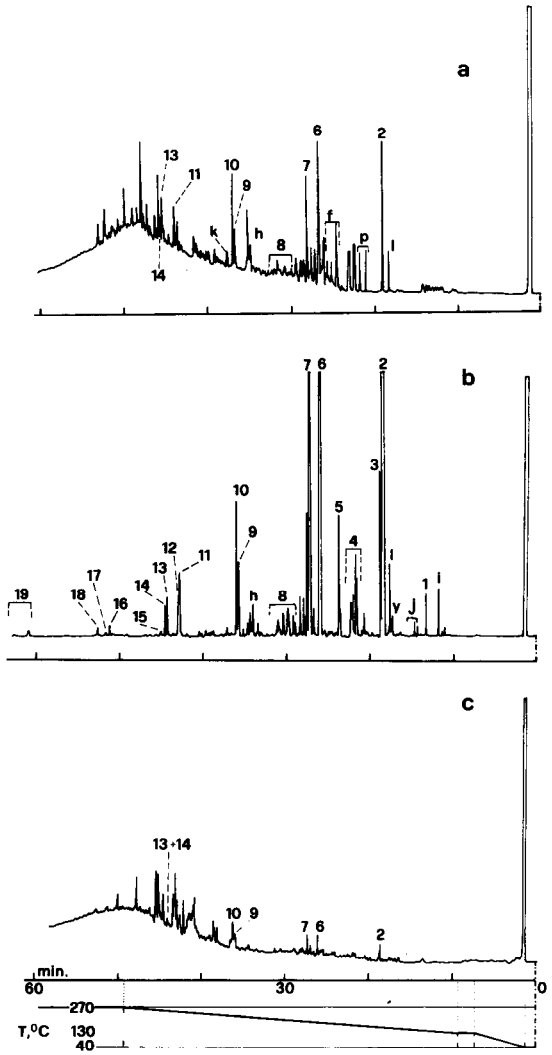

Fig. 3. GC separation of PAHs eluted from the alumina column (CH_2Cl_2 fraction). The letters a–c refer to the same samples as in Fig. 1. Other letters and numbers are used to identify the compounds reported in Table I: 1 = fluorene; 2 = phenanthrene; 3 = anthracene; 4 = methylphenanthrenes/anthracenes; 5 = phenylnaphthalene; 6 = fluoranthene; 7 = pyrene; 8 = methylfluoranthenes/pyrenes; 9 = benzo[*a*]anthracene; 10 = chrysene; 11 = benzo[*k*]fluoranthene; 12 = benzo[*b*]fluoranthene; 13 = benzo[*e*]pyrene; 14 = benzo[*a*]pyrene; 15 = perylene; 16 = indenopyrene; 17 = dibenzanthracene; 18 = benzoperylene; 19 = dibenzopyrenes.

(low-carbon preference index), Fig. 3 shows the wide variability in molecular weight and composition of protomutagenic PAHs present in the three samples. As PAH derivatives present in the dichloromethane eluate represent a minor portion of all these fractions and, in many instances, they exhibit a biological activity very similar to that of PAHs, it is reasonable to believe that possible health effects arising from the dichloromethane fraction are determined mostly by the content of PAHs.

TABLE I

PAHs CONTAINING OXYGEN AND SULPHUR FOUND IN THE CH_2Cl_2 FRACTION ELUTED FROM THE ALUMINA COLUMN

No PAHs containing oxygen and sulphur were detected in the CH_2Cl_2 fraction obtained from the air sample collected in the suburban area.

Type of sample	*No.*	*Compound*	*MW*	*Empirical formula*
Industrial emission	i	Dibenzofuran	168	$C_{12}H_8O$
	j	*x*-Methyldibenzofuran	182	$C_{13}H_{10}O$
	y	9*H*-Fluoren-9-one	180	$C_{13}H_8O$
	l	Dibenzothiophene	184	$C_{12}H_8S$
	h	Benzonaphthothiophene	234	$C_{16}H_{10}S$
Diesel exhaust	l	Dibenzothiophene	184	$C_{12}H_8S$
	p	Methyldibenzothiophenes	198	$C_{13}H_{10}S$
	f	Dimethylnaphthothiophenes	212	$C_{14}H_{12}S$
	h	Benzonaphthothiophene	234	$C_{16}H_{10}S$
	k	Methylnaphthobenzothiophene	248	$C_{17}H_{12}S$

Fig. 4a, b and c show the chromatographic profiles obtained by injecting into the HPLC columns the acetonitrile fractions containing possible direct mutagens. Fig. 4 also shows the subfractions that were collected for qualitative and quantitative purposes. The criteria followed in each case were dictated by the need of making easier the GC and GC–MS identification of the most abundant or toxic PAH derivatives present in each sample. A different approach could be taken if the same subfractions were to be submitted to microbiological assay. Because of the health implications related to the composition of the acetonitrile fractions, the three samples will be discussed in detail.

The first sample considered is the diesel exhaust. As it is known[3] that this type of emission contains substantial amounts of nitro-PAHs, which are strong direct mutagens, the choice of the various cuts was made primarily with the aim of isolating these compounds as efficiently as possible and then of identifying the other PAH derivatives that were present as major components in the mixture. Particularly important, in this respect, was the determination of the components present in the third subfraction in Fig. 4a, which was collected in correspondence with the elution of a sharp peak having a retention equal to that of many nitropyrene and nitrofluoranthene derivatives. Fig. 5a shows the GC profile obtained by analysis of this subfraction. The chromatogram obtained with the flame ionization detector reveals the presence of an intense peak (which accounts for more than 99% by weight of the carbon-containing compounds), followed by other, small components, present at trace levels. By subjecting this subfraction to GC–MS analysis, it was possible to identify positively the major component, which turned out to be 1-nitropyrene. Fig. 5b and c show, respectively, the reconstructed chromatogram obtained at $m/z = 247$ and one of the spectra recorded during the GC–MS analysis.

The other major components that may act as direct mutagens were found in subfractions 4 and 5 and were identified as benzofluorenones, anthracenediones, methylanthracenediones, benzanthrone and 6*H*-benzopyrenediones. Subfractions 6

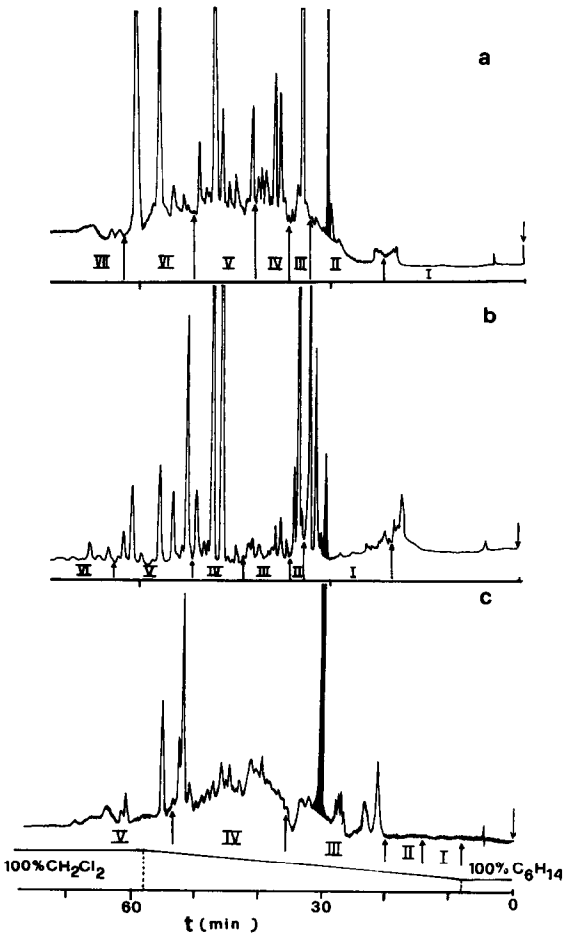

Fig. 4. HPLC separation of the CH_3CN fraction (polar PAHs), collected from the alumina column. Letters a–c refer to the same samples as in Fig. 1. The Roman numbers indicate the subfractions collected and subjected to GC and GC–MS analyses. The shaded peak relates to perdeuteriated nitronaphthalene, added as an internal standard.

and 7 were found to contain mostly phthalates. Other nitro-PAHs present at trace levels were detected by subjecting each subfraction to GC analysis using a nitrogen–phosphorus specific detector. The chromatogram obtained with this method and the nitro-PAHs identified in the sample are shown in Fig. 6. For the sake of clarity, Fig. 6 shows all the subfractions as they were analysed together. As 1-nitropyrene, 9-nitroanthracene and the oxygen-containing PAHs identified represent about 80% of the total mixture and the content of dinitropyrenes is low, it is reasonable to conclude that other minor components together cannot account for more than a few percent of the direct acting mutagenicity, and their identification is of little interest here. Further information on the various oxygen-containing PAHs identified in a diesel exhaust can be found in our previous work carried out on a similar sample[5].

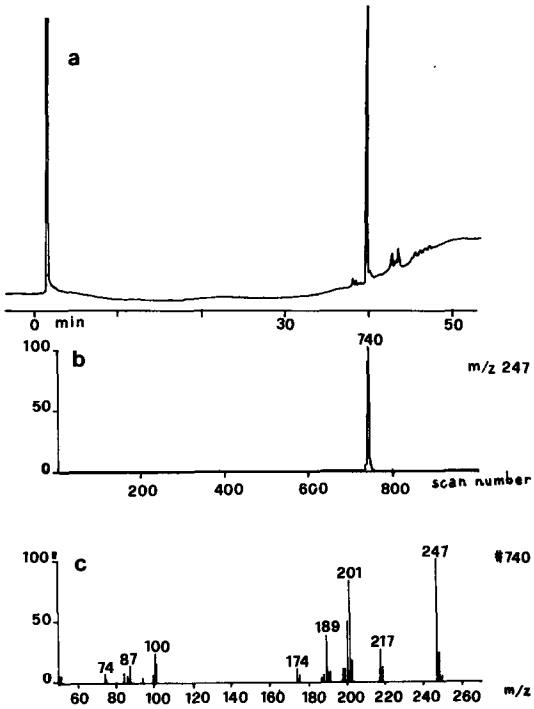

Fig. 5. (a) GC separation of subfraction III in Fig. 4a, collected by HPLC separation of polar PAHs in diesel exhaust emissions. (b) Mass chromatogram at m/z = 247 recorded during the GC–MS analysis of the same fraction as in (a). (c) Mass spectrum of 1-nitropyrene recorded during one of the scans acquired in the GC–MS separation.

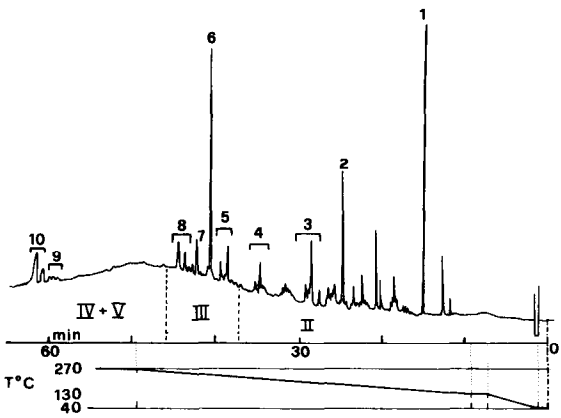

Fig. 6. GC separation of nitro-PAHs found in the diesel exhaust sample. Specific detection was obtained by means of an alkali flame ionization detector. Dotted lines are used to indicate the compounds found in subfractions III, IV and V in Fig. 4a. 1 = 1-Nitronaphthalene-d_7 (internal standard); 2 = 9-nitroanthracene; 3 = methylnitroanthracenes; 4 = trimethylnitronaphthalenes; 5 = nitrofluoranthenes; 6 = 1-nitropyrene; 7 = dinitrofluorene; 8 = methylnitropyrenes; 9 = nitrobenzopyrenes; 10 = dinitropyrenes/fluoranthenes.

The determination of the various components present in the industrial emission was of greater interest, as almost no data are available in the technical literature on this type of source. In order to identify the widest number of PAH derivatives, six subfractions were collected from the HPLC eluate and each was submitted to GC and GC–MS analysis. The GC profiles relative to each subfraction are shown in Fig. 7 and a complete list of the compounds identified is reported in Table II. When positive identification by GC–MS was not possible, the most abundant ions recorded in the mass spectra are reported.

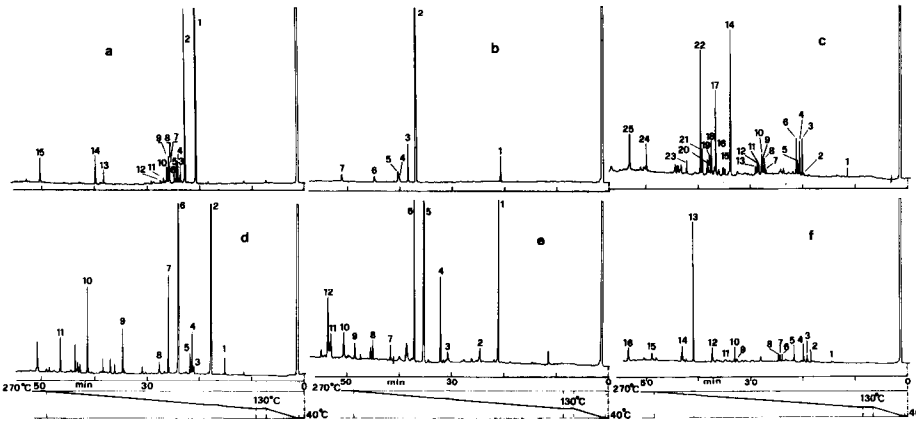

Fig. 7. GC analysis of all subfractions collected by HPLC fractionation of polar PAH samples in an industrial emission. Letters a, b, c, d, e, and f indicate the chromatograms of subfractions I, II, III, IV, V and VI, respectively, shown in Fig. 4b.

The results in Table II indicate that the chemical nature and composition of PAH derivatives found in the industrial emission are different from those measured in the diesel exhaust, as in the former instance the only nitro-PAHs found in the sample (9-nitroanthracene and 7-nitrofluoranthene) were present at trace levels and the only potentially hazardous compounds were PAHs containing carbonyl groups and, to a lesser extent, nitrogen-containing PAHs. These differences can be reasonably explained by the fact that, in diesel engines, particulate matter is formed in the presence of relatively high concentrations of nitrating agents (NO_2, HNO_3, HNO_2), whereas in the industrial process these gases are present at lower levels and oxidation of PAHs volatilized from coke and coal bitumen during the heating process was the preferred route for the production of PAH derivatives.

The last sample, suburban particulate matter from a fairly unpolluted area, is important because the elucidation of the SOF composition can be of great utility in the evaluation of transport phenomena and in assessing the impact of a given anthropogenic emission on a site located far away from the source. The analysis of the subfractions eluted from the HPLC column was consistent with the fact that during sampling (a weekend in May 1985), the emission was low (little traffic in the city and a low use of heating), and only small amounts of particulate matter were transported far away from the city. The subfraction containing nitro-PAHs was extremely minute

TABLE II

POLAR PAHs DETECTED IN THE VARIOUS SUBFRACTIONS COLLECTED BY HPLC ANALYSIS OF THE CH_3CN ELUATE

The compounds were extracted from the particulate matter emitted from an industrial plant. Peak numbers refer to the chromatograms shown in Fig. 7a, b, c, d, e and f, respectively*.

No.	*Compound*	*MW*	*No.*	*Compound*	*MW*
1a	9*H*-Carbazole	167	19c	Methylbenz(anthracen/fluoren)-*x*-one	244
2a	Methylcarbazole	181	20c	Methylbenz(anthracen/fluoren)-*x*-one	244
3a	Methylcarbazole	181	21c	Unknown	264
4a	Methylcarbazole	181	22c	Benz(anthracen/phenanthren)-	
5a	Dimethylcarbazole	195		*x,y*-dione	258
6a	Ethylcarbazole	195	23c	Unknown	253
7a	Dimethylcarbazole	195	24c	Dibenz(anthracen/phenanthren)-*x*-one	280
8a	Dimethylcarbazole	195	25c	Dibenzocarbazole	267
9a	Dimethylcarbazole	195			
10a	Dimethylcarbazole	195			
11a	Dimethylcarbazole	195	1d	Benzophenone	182
12a	Methylethylcarbazole	209	2d	9*H*-Fluoren-9-one	180
13a	*x,y*-Diphenylpyridine	231	3d	Phenanthren-/anthracen-*x*-one	194
14a	*x,y*-Diphenylpyridine	231	4d	Phenanthren-/anthracen-*x*-one	194
15a	3,4,5,6-Dibenzocarbazole	267	5d	Phenanthren-/anthracen-*x*-one	194
			6d	9,10-Anthracenedione	208
			7d	4*H*-Cyclopenta[*def*]phenanthren-	
1b	9*H*-Carbazole	167		4-one	204
2b	Benzocarbazole	217	8d	Methyl(anthracen/phenanthren)-*x,y*-dione	222
3b	Benzocarbazole	217	9d	Benz(anthracen/fluoren)-*x*-one	230
4b	*x,y*-Diphenylpyridine	231	10d	Benz(anthracen/phenanthren)-	
5b	*x,y*-Diphenylpyridine	231		*x,y*-dione	258
6b	Unknown	257	11d	Unknown	269
7b	Dibenzocarbazole	267			
			1e	Xanthone	196
1c	Benzothiazole	135	2e	Unknown	210
2c	Benzoquinoline	179	3e	Unknown	218
3c	9(10*H*)-Anthracenone	194	4e	Coumarin (from PAH with M.W. 202)	220
4c	9*H*-Carbazole	167	5e	Benz(anthracen/fluoren)-*x*-one	230
5c	Benzoquinoline	179	6e	Benz(anthracen/fluoren)-*x*-one	230
6c	Unknown	193	7e**	Unknown	–
7c	Aza(fluoranthene/pyrene)	203	8e	6*H*-Benzo[*cd*]pyren-6-one	254
8c	Aza(fluoranthene/pyrene)	203	9e	Unknown	270
9c	Aza(fluoranthene/pyrene)	203	10e	Indeno(phenanthren/anthracen)-*x*-one	280
10c	Aza(fluoranthene/pyrene)	203	11e	Indeno(phenanthren/anthracen)-*x*-one	280
11c	Aza(fluoranthene/pyrene)	203	12e	Unknown	326
12c	Aza(fluoranthene/pyrene)	203			
13c	Aza(fluoranthene/pyrene)	203			
14c	Benz(anthracen/fluoren)-*x*-one	230	1f	Unknown	169
15c	Cyano(pyrene/fluoranthene)	227	2f	Unknown	185
16c	Cyano(pyrene/fluoranthene)	227	3f	Benzoquinoline	179
17c	Cyano(pyrene/fluoranthene)	227	4f	Benzoquinoline	179
18c	Methylbenz(anthracen/fluoren)-*x*-one	244	5f	Diisobutyl isophthalate	278

(*Continued on p. 462*)

TABLE II (*continued*)

No.	*Compound*	*MW*	*No.*	*Compound*	*MW*
6f	Diisobutyl isophthalate	278	12f	*x*-Hydroxybenzanthracenone	246
7f	Oxo-*x*,*y*-dihydrodibenzoxepine	210	13f	12,14-Dihydro-9,10(3′,4′)-furananthracene-12,14-dione	276
8f	Oxo-*x*,*y*-dihydrodibenzoxepine	210			
9f	Unknown	231	14f**	Unknown	–
10f	Unknown	231	15f**	Unknown	–
11f	Benzothienoquinoline	235	16f	Unknown	279

* Small amounts of a compound, tentatively identified as 7-nitrofluoranthene, and traces of nitroanthracenes were detected in fraction b.

** In the mass spectra of these compounds it was not possible to identify the molecular ion unambiguously.

and only trace of 9-nitroanthracene indicated the presence of anthropogenic impact. The low content of benzofluorenones, anthracenediones, benzanthrone and other oxygenated PAHs, together with the profile of alkanes and PAHs, fully confirmed this observation, as the major emission source in the city at the time was traffic. To appreciate the importance of these results it may be useful to compare the content of nitro-PAHs measured in this sample with that previously obtained during the winter season. As can be seen from Fig. 8a and b, a higher content of 9-nitroanthracene together with the presence of a sharp peak (tentatively identified as a nitropyrene/fluoranthene derivative) was found in the sample collected during winter. The difference observed is in agreement with both the increased amount of emission and the increased number of emission sources during the cold season. This comparison indicates that the sensitivity of the method described here is such that small variations in potentially hazardous direct mutagens, such as those occurring in atmospheric samples collected far from the emission source, can be detected.

As far as the acetonitrile–methanol fractions is concerned, the samples col-

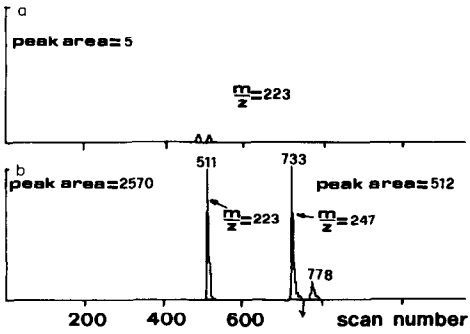

Fig. 8. GC–MS determination of nitro-PAHs present in atmospheric samples collected in a suburban area. (a) Mass chromatogram at m/z = 223 (nitroanthracene/phenanthrene isomers) recorded by injecting subfraction I in Fig. 4c. The sample was collected during a weekend in May 1985. The mass chromatogram at m/z = 247 did not show the presence of 1-nitropyrene. (b) Mass chromatograms at m/z = 223 and 247, recorded during the analysis of the same HPLC subfraction, from a sample of particulate matter collected during winter (a work day in February 1985). The arrow indicates the scan number (740) corresponding to the elution of 1-nitropyrene, which was not detected in this sample. The peak eluted, corresponding to scan 733, was an isomer, tentatively identified as 3-nitrofluoranthene.

lected from the diesel engine and in the atmosphere were characterized by the presence of long-chain polar compounds, certainly members of different homologous series. They were very difficult to identify by GC–MS because of the high intensity of the ions produced by the rupture of the alkyl chain and the low intensity of the molecular ion. In contrast, the composition of the polar fraction in the industrial emission was completely different from the other two, the various compounds present being characterized by a higher aromatic content. However, in this instance also, direct GC or GC–MS analysis appeared to be unsuitable for an unambiguous identification of the various components present in this fraction. Certainly a more systematic approach is necessary for elucidating the composition of the acetonitrile–methanol eluates, but the need for additional chromatographic analyses could only be justified if this fraction is definitely proved to increase the direct mutagenic activity of particulate matter.

PAH derivatives and mutagenic properties of the sample

The results obtained by analysing the SOF extracted from the light-duty diesel exhaust and the industrial emission are important for two reasons: they confirm the presence of nitro-PAHs in diesel particulates and, more important, they suggest that other components, particularly oxygen-containing PAHs, should be considered when evaluating the mutagenic properties of particulate matter.

As, according to the in vitro measurements carried out by Salmeen *et al.*[9], nitro-PAHs present in diesel exhaust account for *ca.* 50–60% of directly acting mutagenicity, and the acetonitrile–methanol fraction that we collected did not contain known direct mutagens in detectable amounts, it is likely that oxygen-containing PAHs present in the acetonitrile fraction may account for the residual mutagenic activity of this sample. The role of oxygen-containing PAHs can be better understood by looking at the results obtained from the analyses of the SOF extracted from the industrial plant. In this instance, the levels of nitro-PAHs were so low that they could not reasonably explain the 30% direct mutagenic activity observed when the concentrated SOF was subjected to microbiological tests. Considering that the contents of PAHs and alkanes in the SOF are 32 and 3% (w/w), respectively, only the residual 15% of the extract can be considered to be directly mutagenic. From Fig. 1b, this portion of the SOF corresponds to the acetonitrile and acetonitrile–methanol fractions, each containing *ca.* 7% (w/w) of total organic material. As, based on the investigations carried out by Cortois *et al.*[12], the most polar fraction extracted from environmental samples usually shows little biological activity, it is reasonable to conclude that most of the directly acting mutagenicity has to be attributed to the acetonitrile eluate, where oxygen-containing PAHs are present in substantial amounts.

Based on the previous considerations, two main conclusions can be drawn. The first is that the chemical characterization of industrial emissions is equally important to that of vehicular emissions in determining the quality of a given atmosphere. The second is that more systematic work should be undertaken to evaluate the direct mutagenic properties of various oxygen-containing PAHs present in the moderately polar fraction. Our results, combined with previous observations made by Ramdhal[13], seem, in fact, to confirm the hypothesis of Pitts *et al.*[9] that oxygen-containing PAHs, particularly keto-PAHs, can be easily formed by oxidation reactions occurring during combustion processes or in the atmosphere. Therefore, the

impact of these components on human health can, under certain conditions, be extremely important.

ACKNOWLEDGEMENTS

We are grateful to Mr. A. Brachetti for technical assistance in the construction of the high-volume sampling shelter. We are indebted to the Mass Spectrometry Service of the Area della Ricerca del CNR, Rome, which provided the MS instrumentation.

REFERENCES

1 M. Moller and I. Alfheim, *Mutat. Res.*, 116 (1983) 35.
2 J. Huising, R. Bradow, R. Jungens, L. Claxton, R. Zwedinger, S. Tejada, J. Bumgarner, F. Duffield, M. Waters, V. F. Simmon, C. Hare, C. Rodrigues and L. M. Snow, in M. Waters, S. Nesnow, J. Huising and L. Claxton (Editors), *Application of Short term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures*, Plenum, New York, 1978, EPA-600/9-78-027.
3 J. N. Pitts, K. A. Van Cauwenberghe, D. Grosjean, J. P. Schmid, D. R. Fitz, W. L. Belzer, G. B. Knudson and P. M. Hynds, *Science*, 202 (1978) 515.
4 T. Ramdahl, K. Kveseth asnd G. Becher, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 19.
5 A. Liberti, P. Ciccioli, E. Brancaleoni, C. di Palo and A. Cecinato, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 601.
6 M. Oehme, S. Mano and H. Stray, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 417.
7 T. Ramdahl and K. Urdal, *Anal. Chem.*, 54 (1982) 2256.
8 M. C. Paputa-Peck, R. S. Marano, D. Schuetzle, T. L. Riley, C. V. Hampton, T. J. Prater, L. M. Skewes, T. E. Jensen, P. H. Ruehle, L. C. Bosch and W. P. Duncan, *Anal. Chem.*, 55 (1983) 1946.
9 I. T. Salmeen, A. M. Pero, R. Zator, D. Schuetzle and T. L. Riley, *Environ Sci. Technol.*, 18 (1984) 375.
10 T. H. Risby and S. S. Lestz, *Environ Sci. Technol.*, 17 (1983) 621.
11 M. Katz, (Editor), *IARC Scientific Publication No. 19, Environmental Quality*, York University, Downsview, 1978, pp. 206–213.
12 Y. A. Cortois, A. Person, Y. Le Moullec, F. Coviaux and B. Festy, *Proceedings of VIth World Congress on Air Quality (IUAPPA), 16–20 May 1983, Port Maillot, Paris*, Vol. 2, Sepic Publishers, Paris, pp. 113–120.
13 T. Ramdahl, *Environ Sci. Technol.*, 17 (1983) 666.