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Application of the *Salmonella* Mutagenicity Assay and Determination of Polycyclic Aromatic Hydrocarbons in Workplaces Exposed to Petroleum Pitch and Petroleum Coke

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Summary. Workplaces of an Italian carbon electrode factory, exposed to petroleum pitch and petroleum coke, were studied using a coupled chemical and biological approach to evaluate occupational mutagenic/carcinogenic hazards. Analytical procedures for the determination of polycyclic aromatic hydrocarbons (PAH) and *Salmonella*/microsome mutagenicity tests (with TA98 and TA100 strains) were performed on both industrial ingredients (pitch and coke) and airborne particulate matter of the working environment, after fractionating by sequential Soxhlet extractions with four organic solvents of increasing polarity (benzene, chloroform, methanol and acetone). The results showed: (a) the presence of extraordinarily high PAH (carcinogenic and non-carcinogenic) contents in the benzene extracts of petroleum pitch (3.6 wt% of total PAH) and of airborne particulate samples (up to 0.35 wt% of total PAH), in correlation with very high indirect (after metabolic activation) mutagenic responses of benzene extracts with strain TA98; (b) very high indirect mutagenic responses in the other extracts of the airborne particulate samples (especially with strain TA98); (c) the production during the processing at high temperatures of directly acting mutagens (without metabolic activation) which were absent in the starting materials and their release in the air of workplaces. The comparison of chemical analytical and mutagenicity data has proved to be an interesting approach for better defining the relative health hazards due to occupational exposure to potentially mutagenic/carcinogenic petroleum products.

Key words: Occupational mutagens and carcinogens – Salmonella test – Polycyclic aromatic hydrocarbons – Petroleum pitch – Carbon electrode industry

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The extremely wide range of industrial processes exposed to mutagenic/carcinogenic substances indicates the need for applying short-term screening tests to identify occupational hazards in work environments. At present, there exists a great deal of research on the application of short-term mutagenicity tests to evaluate the mutagenic properties of single chemicals as predictive of potential carcinogenicity. Less research has been performed on samples actually encountered in occupational and environmental exposures, where often highly complex chemical mixtures of uncertain composition and effects on health are present. Recently, the *Salmonella/*microsome test [2], one of the most used and most valid short-term mutagenicity bioassays, has been successfully applied by some authors and ourselves, to environmental mixtures such as air and water pollutants [7, 8, 24, 28, 33, 38], cigarette smoke condensate [9, 27, 36], body fluids [12, 23], and to samples present in work environments, such as airborne particulate samples and industrial materials [10, 11, 17, 25]. Even if there is still a lack of data on the application of this test in work environments, this bioassay appears to be promising as an early warning system for monitoring industrial environments for the presence of mutagenically active substances, because of its correlation degree between the carcinogenicity of compounds and their mutagenic potential, its low cost and rapid response [1, 3, 4, 6, 35, 41].

Among the numerous work environments known to be exposed to carcinogenic risks, the industrial processes where petroleum products are handled and produced deserves particular attention [5]. Carcinogenic or cocarcinogenic constituents have been discovered in these products: for instance, a number of authors [5, 14–16, 20, 22, 29, 30, 40] have studied the presence of polycyclic aromatic hydrocarbons (PAH), some of which are known to be carcinogenic or cocarcinogenic [3, 18–20], in work operations with mineral oils, tar, pitch or asphalt handling and production, and petroleum distilling [5, 14–16, 20, 22, 29, 30, 40]. According to Guerin [15] "operations accompanied by the greatest hazard of occupational carcinogenesis are those involving contact with petroleum residuals and respiration of heavily PAH-contaminated environments". Therefore, there is an increasing necessity to have detailed knowledge of PAH content in these work environments exposed to petroleum products to evaluate health hazards.

In the present paper, we have studied, using a multicentered approach, the applicability and significance of the *Salmonella/*microsome test [2] along with the monitoring of PAH presence in evaluating potential cancer hazards associated with industrial processes with exposure to petroleum products. For our research we chose the work environment of a carbon electrode factory in central Italy, where potentially carcinogenic products, such as petroleum coke, petroleum pitch and coal tar pitch are used. Petroleum pitch is a widely used industrial product and has a high carcinogenic potential, because it has been demonstrated that it is a kind of a petroleum product heavily enriched in PAH content [14].

Our research programme was carried out contemporarily using a coupled analytical-biological approach in different directions:

1. By a *survey in the working environment,* studying by chemical analyses and mutagenicity tests different environmental samples, such as the materials used by workers and airborne particulate samples:

2. by a *survey of the workers,* studying the presence of mutagenetically active metabolites in the urine of exposed workers and comparing these results with the environmental survey.

The present paper only deals with the study of the working environment. The data obtained from the survey of the urine samples are still under elaboration and will be the object of another paper.

Materials and Methods

1. Working Environment

The factory under study employed over 1000 male workers and produced carbon electrodes utilizing two industrial ingredients with potential mutagenic/carcinogenic properties: petroleum coke and, especially, petroleum pitch or coal tar pitch. These products are mixed and then undergo two thermal processes, the first at about 1000° C and the second at about 3000° C (graphitization process). In the factory there is a research laboratory with a pilot plant, where the entire industrial process is reproduced and where the environmental exposure appeared particularly high. We chose for our working environment survey two exposed workplaces:

Workplace A, a pilot plant, where the entire process is reproduced and at the time of the present research petroleum coke and pitch were used;

Workplace B, a laboratory, where dilatation tests at high temperature (about 2600° C) on non-finished electrodes, produced in Workplace A, are performed.

The scheme of the coupled chemical and biological procedures followed for monitoring polycyclic aromatic hydrocarbons and mutagens in these workplaces is illustrated in Fig. 1.

2. Collection and Analysis of Petroleum Coke and Petroleum Pitch Samples

Petroleum coke and petroleum pitch samples were collected and analysed for weights per cent of extractables by different solvents, for PAH content and finally for mutagenic properties. These last two determinations will be described in sections four and five respectively.

Determination of the Weights per Cent of Extractables by Some Solvents. Coke and pitch samples were ground in a mortar. Pitch samples (10 g) and coke samples (50 g) were placed in cellulose thimbles and, then, sequentially submitted to four extractions with increasing polar organic solvents (spectrograde, C. Erba)—benzene, chloroform, methanol and acetone—in a Soxhlet apparatus, for 8 h with 250 ml for each solvent. The benzene-soluble fractions were weighed to constant weight and then divided into two equal parts: one half was used for the PAH analysis and the other for the mutagenicity test.

Preparation of Test Materials for Mutagenicity Assay. The four fractions of each sample were dried, weighed, dissolved in dimethylsulfoxide (DMSO) and tested at different concentrations by the *Salmonella/*microsome test described in section five. The black powders, remaining after the four sequential extractions of coke and pitch samples, were weighed and tested as DMSO suspensions in the 0.1–4 mg/plate range by the same assay [25].

3. Airborne Particulate Sampling and Analysis

The assays performed on airborne particulate samples collected in Workplace A and Workplace B were the determination of time-weighted-average (TWA) exposures to suspended particulate, the determination of the weights per cent of extractables by some organic solvents, the determination of PAH content and the mutagenicity tests. These last two determinations are described in sections four and five.

Determination of TWA Exposures to Suspended Particulate and to Benzene Extractable Matter. The 8 h time-weighted average (8 h-TWA) exposures to suspended particulate (expressed as mg/m^3) in

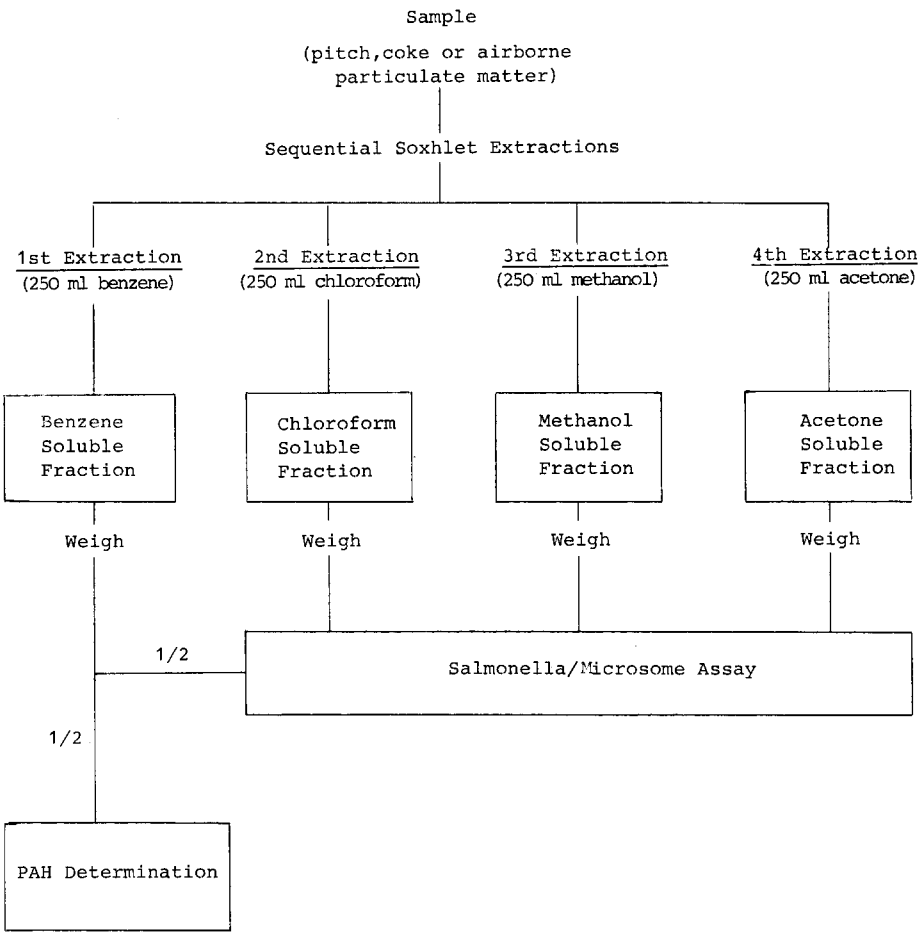

Fig. 1. Scheme of chemical and biological procedures for determining polycyclic aromatic hydrocarbons *(PAH)* and mutagenic activity in petroleum pitch and coke, and in airborne particulate matter of carbon electrode workplaces

the two workplaces were determined by drawing air through 37-mm membrane filters (0,45 μm pore-size, Gelman DM-450 Metricel), placed in the breathing zone of the workers, by means of a sampler pump (1.2 m^3/h flow rate) and weighing the collected particulate. The loaded membrane filters were Soxhlet extracted with 100 ml benzene for 8 h. The benzene soluble fraction was dried, the residue weighed to constant weight (the residue of a clean filter extracted with 100 ml benzene was subtracted). The TWA concentrations of suspended particulate and of benzene soluble substances (expressed as mg/m^3) were the mean of five 8 h TWA determinations carried out during a 40 h working week.

Determination of the Weights per Cent of Extractables by Some Solvents. Airborne particulate samples, for determination of wt% extractable PAH determination and mutagenicity assays, were collected in the two workplaces by drawing air through glass fiber filters (274 cm^2, S & S, Selecta n. 9) using high volume samplers (Staplex). The sampling time for each filter was 8 h and the total sampling time for the two workplaces were equivalent to two workshifts (2 × 8 h). The loaded glass fiber filters were weighed at constant weight (the total suspended particulate was usually about 500 mg) and were first Soxhlet extracted with 250 ml benzene for 8 h in cellulose thimbles. The benzene soluble fractions were filtered, dried, weighed at constant weight (blank subtracted).

The residues were dissolved in benzene and then divided into two equal parts. As in the case of coke and pitch samples, one half was used for PAH determination and the other for mutagenicity assays. The filters were succesively Soxhlet extracted in sequence with 250 ml of three other organic solvents (chloroform, methanol and acetone).

Preparation of Test Materials for Mutagenicity Assays. The four solvent soluble fractions, filtered, dried and weighed, were dissolved in DMSO and tested at different concentrations by the *Salmonella/*microsome assay described in section five. The black powder remaining on the filters after the four extractions were scraped off, ground in a mortar and weighed; mutagenicity assays were performed on DMSO suspensions containing from 0.1 to 4 mg/plate of these insoluble samples [25].

4. Polycylic Aromatic Hydrocarbons Determination

Half the portion of each benzene extract of pitch, coke and airborne particulate samples was added, before determining the PAH content, to a known amount of tritiated benzo(a)pyrene (0.052 μCi) as an internal standard, for the per cent recovery determination, and was submitted to an analytical procedure which was based on our previous works [29, 30] and consisted of the following steps.

PAH Preseparation by Thin-layer Chromatography. Plates precoated with a 2 mm layer of silica gel (Merck) were used for PAH preseparation. The benzene extracts were applied on plates as lines. A mixture of standard PAH was spotted near the benzene extracts on each plate for marking the PAH area. The chromatograms were developed with a mixture of cyclohexane-benzene (1:1). The corresponding PAH areas of extracts were singled out by two UV lamps (254 and 366 nm); standards and extracts were marked and the R_f values recorded. The absorbent of each marked area was scraped off, ground and extracted in a small sintered glass filter tunnel, first with 60 ml of benzene and then with 60 ml of dichloromethane.

Thin-layer Chromatographic Separation of PAH. Plates precoated with a 0.5 mm layer of 20% acetylated cellulose plate (20 Acetil CE, C. Erba) were used. The extracts of PAH areas from silica gel plates were applied on the plates as lines and the chromatograms were developed by a mixture of ethanol-dichloromethane-water (20:10:1). The single fluorescent lines were scraped off and extracted in small sintered glass filter funnels, first with 40 ml of benzene and then with 40 ml of ethyl ether.

PAH Determination by Spectrophotofluorometry and Spectrometry. The benzene extracts of each fluorescent line were dried using a rotary vacuum evaporator at 37° C and taken up with specific volumes of spectrograde cyclohexane. These dilute solutions were analysed by spectrophotofluorometry using an Aminco-Bowman spectrophotofluorometer with cells of 1-cm path, a photomultiplier tube 1P21 and Xenon lamp; slit arrangements: 0.5 mm. The wavelengths used for recording emission and excitation spectra for qualitative and quantitative estimations were as according to previous works [29, 37]. Calibration curves of pure PAH were determined under the same conditions as those encountered in the analysis. The qualitative estimation was also confirmed by ultraviolet and visible spectrometry.

5. Mutagenicity Assay with the Salmonella/Microsome Test

Bacterial Strains. Four strains of *Salmonella thyphimurium* were used preliminarily for the mutagenicity tests. TA98 and TA1538, which are considered to be frameshift mutants and should detect frameshift mutagens, and TA100 and TA1535, which are presumed to detect base substitution mutagens (as noted by some authors [26], the response to TA 100 is somewhat ambiguous since it responds to mutagens of both types). These preliminary tests indicated TA100 and TA98 to be the most sensitive strains, so they were used for the routine and complete assay. All the bacterial strains were supplied by Prof. C. Monti-Bragadin, University of Trieste, Italy, and were from Dr. B. N. Ames, University of California, Berkeley.

Metabolising System. Intraperitoneal injections of Aroclor 1254 in Sprague-Dawley male rats were used to induce rat liver enzyme systems. Liver microsome and S9 mix were prepared according to the Ames method [2].

Test Procedure. The weighed extracts from the four sequential extractions of coke, pitch and airborne particulate samples were dissolved in dimethylsulfoxide (DMSO). The remaining non-extractable residues were weighed and DMSO suspensions were prepared [25]. Then, extracts and residues were tested in duplicate in dose response curves for mutagenic properties by the plate incorporation tests, with and without metabolic activation systems (rat liver S9 mix), following the Ames et al. technique [2].

For interpreting results and for comparing the mutagenic potentials of different fractions of the samples under study, we computed the so-called *mutagenic activity ratio* [6, 8, 13], as follows:

$$\text{Mutagenic Activity Ratio} = \frac{E - C}{C}$$

Where: E = mutagenic activity of fraction (revertants/plate);
C = control value of blank on the day of the test (spontaneous revertants/plate).

Controls. Appropriate positive and negative controls in duplicate as well as blanks, consisting of new thimbles and filters extracted with the four organic solvents, were included in each assay. Routine controls for sterility of samples, S9 mix, and reagents were also carried out.

Interpretation of Results. The criteria for positive results were the observations of a dose-related response and of a mutagenic activity ratio 2 or higher, i.e. a three-fold or greater number of revertants in experimental tests than in the control tests [2, 11, 13].

Results

1. Results of Chemical Analysis and Mutagenicity Tests on Pitch and Coke Samples

Table 1 shows the weights per cent of extractable substances obtained from coke and pitch samples by extraction with the four organic solvents. The coke sample

Table 1. Quantity found (%) of solvent soluble fractions and residues from coke and pitch samples

Fractions and residues	Quantity found (%)[a]	
	Petroleum coke	Petroleum pitch
Benzene soluble fraction	0.10	35.11
Chloroform soluble fraction	0.06	22.48
Methanol soluble fraction	0.12	3.83
Acetone soluble fraction	0.01	1.33
Extractable (sum of all fractions)	0.29	62.75
Non-extractable (residue)	99.71	37.25

[a] Obtained by 8 h Soxhlet extractions, sequentially with 250 ml of each solvent (weight of solvent blank subtracted)

Table 2. Polycyclic aromatic hydrocarbons (PAH) content of coke and pitch samples (μg/g)

Polycyclic aromatic hydrocarbons	Concentration (μg/g)[a]	
	Petroleum coke	Petroleum pitch
Antanthrene[b]	N.D.	2,250
Benzo(b)fluoranthene[b]	0.004	N.D.
Benzo(k)fluoranthene	0.001	1,140
Benzo(a)pyrene[b]	0.040	16,540
Fluoranthene	0.188	69
Perylene	0.082	18,750
Total PAH content	0.315	38,749

[a] The values are the mean of two determinations and were corrected for the losses by adding tritiated benzo-(a)pyrene as an internal standard

[b] Carcinogenic hydrocarbon [3, 19, 20]

N.D. = not detectable

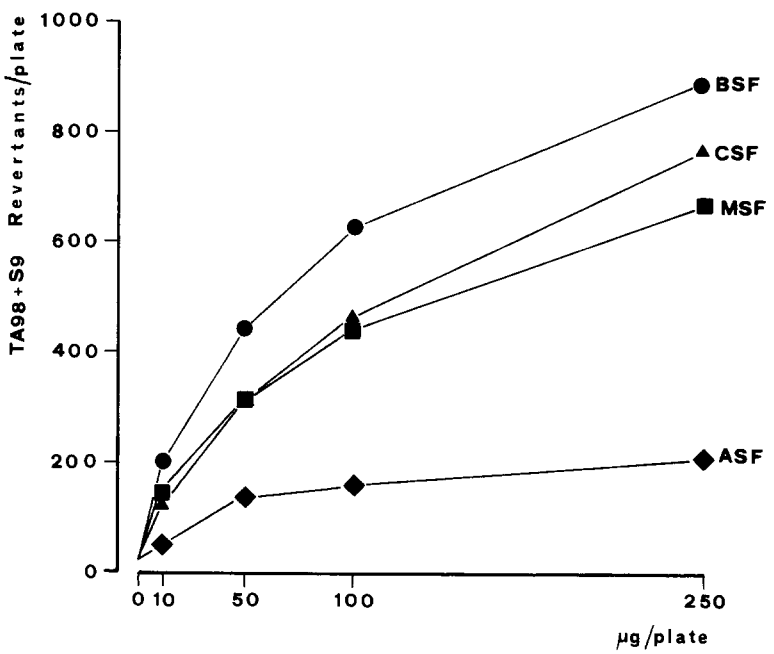

Fig. 2. Dose response curves for mutagenicity of solvent soluble fractions from petroleum pitch sample, with strain TA98 and metabolic activation (+S9 mix). Benzene soluble *(BSF)*, chloroform soluble fraction *(CSF)*, methanol soluble fraction *(MSF)* and acetone soluble fraction *(ASF)*

contained a very low percentage of total extractable fractions (0.29 wt%), whereas the pitch sample yielded a very high percentage (62.75 wt%), with a maximum value (35.1 wt%) for benzene soluble fraction.

Table 3. Mutagenic response with TA98 strain and metabolic activation (+S9 mix) of solvent soluble fractions and residue of petroleum pitch sample

Soluble fractions and residue	Amount per plate (μg)	Mutagenic response with TA98 strain (+S9)[a]	
		Revertants/plate	Mutagenic activity ratio[b]
Benzene soluble fraction	10	204	7.2
	50	444	16.8
	100	624	23.9
	250	888	34.5
Chloroform soluble fraction	10	125	4.0
	50	310	11.4
	100	468	17.7
	250	768	29.7
Methanol soluble fraction	10	147	4.8
	50	312	11.5
	100	410	15.4
	250	668	25.7
Acetone soluble fraction	10	50	1.0
	50	130	4.2
	100	156	5.2
	250	220	7.8
Non-extractable (residue)	100	—	—
	500	—	—
	1,000	—	—

[a] Control strain was: 25 rev/plate

[b] Mutagenic activity ratio = $\frac{E-C}{C}$; where: E = revertants/plate for the test fraction; C = control value of spontaneous revertants/plate on the day of the test

"—" = negative results

Determination of Polycyclic Aromatic Hydrocarbons in Benzene Fractions. Table 2 gives the results, corrected for the losses by internal standard (tritiated benzo(a)-pyrene), of PAH determination in benzene-soluble fractions of coke and pitch samples. The total PAH content of the coke sample was low (0.31 μg/g) in comparison to that of the pitch sample, which contained a very high level of PAH (38.74 mg/g, that is to say 3.8 wt%)[1]. Furthermore, high quantities (2.25 mg/g) of a carcinogenic and mutagenic PAH, antanthrene [3], were determined in the pitch sample. The well-known carcinogenic and mutagenic PAH, benzo(a)pyrene, was determined in both samples, but the pitch contained a very high quantity of this carcinogen (about 1.6 wt%), whereas in the coke sample only trace levels of this carcinogen (0.04 μg/g) were found together with the carcinogenic benzo(b)fluor-anthene (0.004 μg/g).

1 Greinke and Lewis [14], analysing petroleum pitch samples, found a PAH content of almost 2.5 wt%

Mutagenicity Assay with Salmonella/Microsome Test. No mutagenic activity was observed for the coke fractions and residue with strains TA98 and TA100 (with and without metabolic activation), in relationship to the low PAH content found. As expressed in the dose response curves (Fig. 2) and in the results of Table 3, the four solvent soluble fractions of the pitch sample showed very high mutagenic activity only when using strain TA98 (frameshift mutations) and exclusively with metabolic activation (+S9 mix). All tested quantities of the benzene soluble fraction had the strongest mutagenic activity, but also the chloroform and methanol soluble fractions showed a very high activity with TA98 plus S9 mix. The acetone soluble fraction was mutagenic but at a much lower level than the other three fractions. The non-extractable residue of pitch sample was not mutagenic.

Comparing the results of chemical analysis and mutagenicity assays shown in Tables 1, 2, 3, and in Fig. 2, it is possible to observe that in the benzene soluble fraction of the pitch sample, the presence of a high quantity of mutagenic/carcinogenic PAH corresponds to a very high mutagenic activity response using TA98 strain only with metabolic activation.

2. *Results of Chemical Analysis and Mutagenicity Tests on Airborne Particulate Samples of Workplaces*

Time-Weighted Average (TWA) Concentration of Total Suspended Particulate and Benzene Extractables. The 8 h time-weighted average (8 h-TWA) concentrations of total suspended particulate, expressed as mean values of a working week (8 h × 5 days), were 0.66 mg/m^3 (range 0.45–0.99) in Workplace A and 0.57 (range 0.23–1.00) in Workplace B. The environmental exposures to benzene extractables[2] were 0.05 mg/m^3 for Workplace A and 0.06 mg/m^3 for Workplace B.

Weight per Cent of Solvent Soluble Fractions for Airborne Particulate Samples. Table 4 gives the weights per cent of soluble substances extracted from airborne particulate samples of the two workplaces. These values are similar, even if the wt% of the total extractable matter was slightly lower in Workplace A than in Workplace B (16.9% vs. 22.6%).

Determination of Polycyclic Aromatic Hydrocarbons. Table 5 shows the results of PAH content of airborne particulate samples of the workplaces. The total PAH content and carcinogenic PAH (antanthrene and benzo(a)pyrene) content of Workplace A sample were very high: 0.36 wt% of total PAH and 0.10 wt% of carcinogenic PAH (0.09 wt% of benzo(a)pyrene). In the Workplace B sample, on the contrary, a very low total PAH content (0.02 wt%) and only one carcinogenic PAH (benzo(a)pyrene) were present.

Mutagenicity Assays with Salmonella/Microsome Test. Figures 3–8 give the dose response curves of mutagenic activity of solvent soluble fractions obtained from Workplace A and B samples on strains TA98 and TA100, both with and without metabolic activation. The number of revertants/plate obtained at different doses and the mutagenic activity ratio of the extracts and residues of the two samples on these strains are reported in Table 6.

2 In USA, the federal limit in work environments for coal tar products is 0.2 mg/m^3 of benzene soluble matter [31, 32]

Table 4. Quantity found (%) of solvent soluble fractions and residues from airborne particulate samples

Fractions and residues	Quantity found (%)[a]	
	Workplace A	Workplace B
Benzene soluble fraction	8.30	11.90
Chloroform soluble fraction	1.99	2.50
Methanol soluble fraction	6.10	7.43
Acetone soluble fraction	0.55	0.73
Extractable (sum of all fractions)	16.94	22.56
Non-extractable (residue)	83.06	77.44

[a] Obtained by 8 h Soxhlet extractions sequentially with 250 ml solvent (weight of solvent blank subtracted)

Table 5. Polycyclic aromatic hydrocarbons (PAH) content of airborne particulate samples collected in the two workplaces (μg/g)

Polycyclic aromatic hydrocarbons	Concentration (μg/g)[a] in airborne particulate	
	Workplace A	Workplace B
Antanthrene[b]	128.94	N.D.
Benzo(k)fluoranthene	287.53	54.23
Benzo(a)pyrene[b]	908.00	33.67
Fluoranthene	670.44	60.64
Perylene	1,597.64	45.69
Total PAH content	3,592.55	194.23

[a] The values are the mean of two determinations and were corrected for the losses by adding tritiated benzo-(a)pyrene as an internal standard

[b] Carcinogenic hydrocarbon [3, 19, 20]

N.D. = not detectable

Almost all the extracts of both samples showed high mutagenic activity on the TA98 strain either with or without metabolic activation, whereas only the benzene soluble fractions were mutagenic on the TA100 strain with and without metabolic activation. *Response on TA98 strain.* All tested quantities of both benzene extracts showed very high indirect mutagenic responses and always the highest among the responses of the other extracts. Comparing the two samples, the values of mutagenic activity ratio with activation were, at the same concentration, much

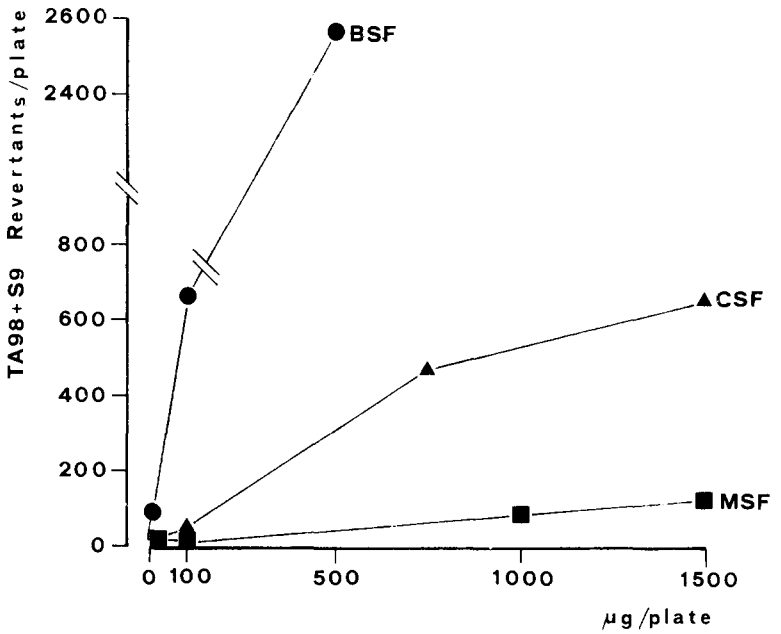

Fig. 3. Workplace A: dose response curves for mutagenicity of solvent soluble fractions from airborne particulate sample, with strain TA98 and metabolic activation (+S9 mix). Benzene soluble fraction *(BSF)*, chloroform soluble fraction *(CSF)* and methanol soluble fraction *(MSF)*

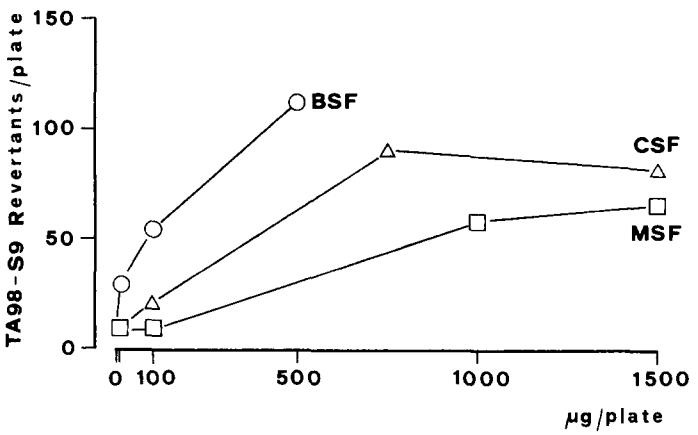

Fig. 4. Workplace A: dose response curves for mutagenicity of solvent soluble fractions from airborne particulate sample, with strain TA98 and without metabolic activation (−S9 mix). Benzene soluble fraction *(BSF)*, chloroform soluble fraction *(CSF)* and methanol soluble fraction *(MSF)*

higher for Workplace A than for Workplace B. The chloroform soluble fractions were also very mutagenic but at a much higher concentration than benzene extracts. Methanol and acetone fractions of each sample were mutagenic but at a much lower level than the other fractions. The mutagenic response without

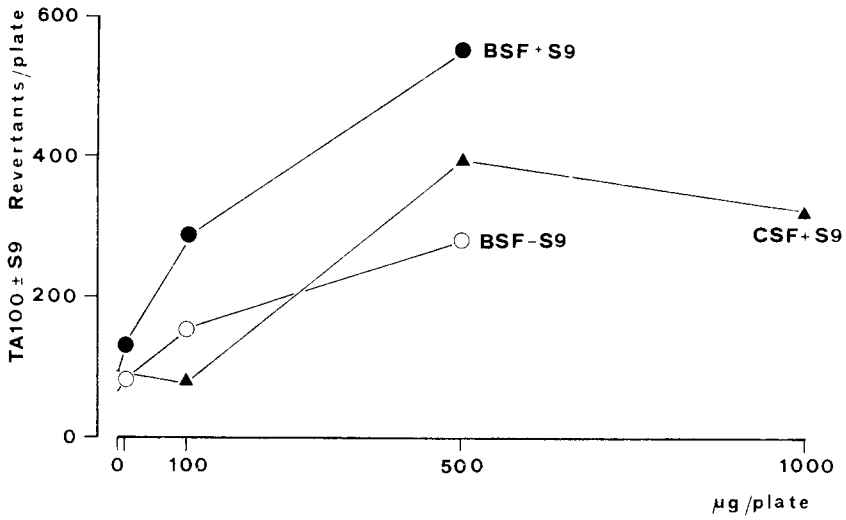

Fig. 5. Workplace A: dose response curves for mutagenicity of solvent soluble fractions from airborne particulate sample, with strain TA100, with or without metabolic activation (± S9 mix). Benzene soluble fraction *(BSF + S9)*, chloroform soluble fraction *(CSF + S9)* and benzene soluble fraction *(BSF − S9)*

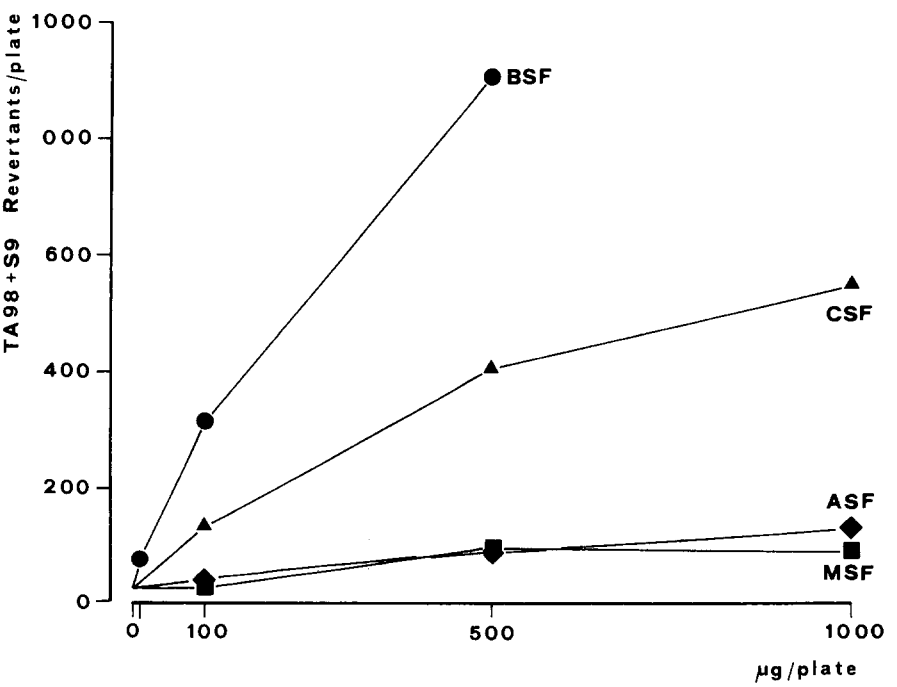

Fig. 6. Workplace B: dose response curves for mutagenicity of solvent soluble fractions from airborne particulate sample, with strain TA98 and metabolic activation (+S9 mix). Benzene soluble fraction *(BSF)*, chloroform soluble fraction *(CSF)*, methanol soluble fraction *(MSF)* and acetone soluble fraction *(ASF)*

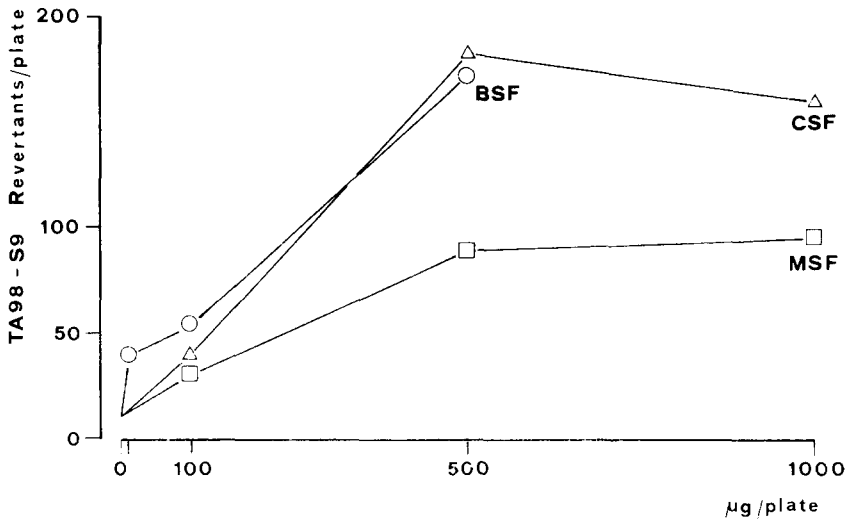

Fig. 7. Workplace B: dose response curves for mutagenicity of solvent soluble fractions from airborne particulate sample, with strain TA98 and without metabolic activation (−S9 mix). Benzene soluble fraction *(BSF)*, chloroform soluble fraction *(CSF)* and methanol soluble fraction *(MSF)*

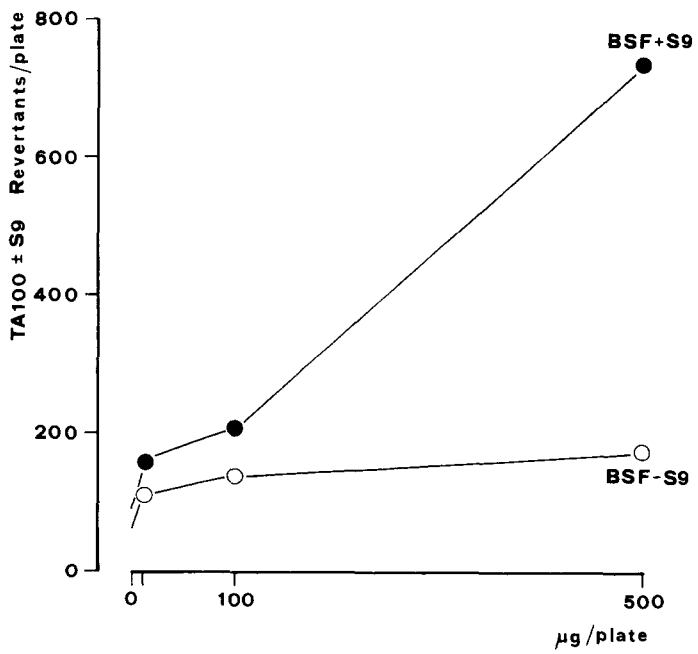

Fig. 8. Workplace B: dose response curves for mutagenicity of benzene soluble fractions from airborne particulate sample, with strain TA100, with or without metabolic activation (± S9 mix)

Table 6. Mutagenic response with TA98 and TA100 strains of solvent soluble fractions and residues of airborne particulate samples

Soluble fractions and residue	Amount per plate (μg)	Mutagenic response with TA98 strain[a]				Mutagenic response with TA100 strain[b]			
		Revertants/plate		Mutagenic activity ratio[c]		Revertants/plate		Mutagenic activity ratio[c]	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Workplace A									
Benzene	10	29	97	1.6	3.8	81	136	0.1	0.5
soluble	100	55	664	4.0	32.2	157	290	1.2	2.3
fraction	500	114	2,576	9.4	127.8	281	552	2.9	5.2
Chloroform	100	22	54	1.0	1.7	—	82	—	0.1
soluble	750	91	470	7.3	22.5	—	400	—	3.5
fraction	1,500	82	656	6.5	31.8	—	324	—	2.6
Methanol	100	13	12	0.2	0.4	—	—	—	—
soluble	1,000	59	96	4.4	3.8	—	—	—	—
fraction	1,500	66	122	5.0	5.1	—	—	—	—
Acetone	10	—	48	—	1.4	—	—	—	—
soluble	100	—	55	—	1.8	—	—	—	—
fraction	500	—	108	—	4.4	—	—	—	—
Non-	100	—	—	—	—	—	—	—	—
extractable	500	—	—	—	—	—	—	—	—
(residue)	1,000	—	—	—	—	—	—	—	—
Workplace B									
Benzene	10	41	80	2.7	2.2	59	160	0.0	0.7
soluble	100	55	316	4.0	11.6	140	208	1.4	1.2
fraction	500	174	916	14.8	35.6	176	734	2.1	6.8
Chloroform	100	41	134	2.7	4.4	—	—	—	—
soluble	500	184	410	15.7	15.4	—	—	—	—
fraction	1,000	160	556	13.5	21.2	—	—	—	—
Methanol	100	32	29	1.9	0.2	—	—	—	—
soluble	500	90	98	7.2	2.9	—	—	—	—
fraction	1,000	96	90	7.7	2.6	—	—	—	—
Acetone	100	—	41	—	0.6	—	—	—	—
soluble	500	—	91	—	2.6	—	—	—	—
fraction	1,000	—	128	—	4.1	—	—	—	—
Non-	100	—	—	—	—	—	—	—	—
extractable	500	—	—	—	—	—	—	—	—
(residue)	1,000	—	—	—	—	—	—	—	—

[a] Control strain was: Workplace A = 11 rev/plate without S9; 20 rev/plate with S9. Workplace B = 11 rev/plate without S9; 25 rev/plate with S9

[b] Control strain was: Workplace A = 72 rev/plate without S9; 98 rev/plate with S9. Workplace B = 58 rev/plate without S9; 94 rev/plate with S9

[c] See footnote to Table 3

"—" = negative results

metabolic activation was moderately high in benzene, chloroform and methanol fractions of both samples, showing the presence of directly acting mutagens, which were absent in the starting materials (coke and pitch). Presumably the processing at high temperatures changed the composition of the raw materials. It can be observed that all the tests without metabolic activation gave results contrary to those obtained with activation: Workplace B fractions always showed a higher mutagenic activity ratio. *Response on TA100 strain.* The benzene extract of Workplace A sample showed activity both with activation and without activation. Workplace B sample showed a similar response. However, the mutagenic activity ratio of each sample, with and without activation, was very low.

Discussion

The coupled chemical and biological approach followed in the present work proved to have a good applicability for monitoring the presence of PAH and other mutagenic compounds in work environments exposed to hazardous petroleum products. The sequential extraction procedure with increasing polar organic solvents permitted the study of the mutagenic potential of different fractions. In particular, this research provided the following results:

a) The presence of very high PAH (carcinogenic and non) contents in benzene extracts of petroleum pitch and airborne particulate samples and very low PAH content in coke sample;

b) a correlation between PAH content and mutagenic response for benzene extracts of all the samples (especially with TA98 strain and metabolic activation);

c) the presence of mutagenic substances also in chloroform, methanol and acetone extracts (especially with TA98);

d) an enhancement of the indirect (with metabolic activation) mutagenic response in the airborne particulate samples in comparison with the starting materials;

e) the production, during the processing at high temperatures, of directly acting mutagens and their release in the workplaces;

f) a scarse reliability of measurements of TWA concentration for total suspended matter and benzene soluble matter in evaluating occupational exposure to mutagens/carcinogens.

In conclusion, the intercomparison of chemical and mutagenicity data has proved to be a valid approach for better defining the relative health hazard due to occupational exposure to petroleum products or other industrial materials, potentially containing carcinogens and/or mutagens.

The research of mutagenic metabolites in urine samples of exposed workers of Workplaces A and B, still under elaboration, will be compared with the present results and will complete our research programme.

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