DNA single strand breakage, DNA adducts, and sister chromatid exchange in lymphocytes and phenanthrene and pyrene metabolites in urine of coke oven workers

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Abstract

***Objectives*—To investigate the specificity of biological monitoring variables (excretion of phenanthrene and pyrene metabolites in urine) and the usefulness of some biomarkers of effect (alkaline filter elution, ^{32}P postlabelling assay, measurement of sister chromatid exchange) in workers exposed to polycyclic aromatic hydrocarbons (PAHs).**

***Methods*—29 coke oven workers and a standardised control group were investigated for frequencies of DNA single strand breakage, DNA protein cross links (alkaline filter elution assay), sister chromatid exchange, and DNA adducts (^{32}P postlabelling assay) in lymphocytes. Phenanthrene and pyrene metabolites were measured in 24 hour urine samples. 19 different PAHs (including benzo(a)pyrene, pyrene, and phenanthrene) were measured at the workplace by personal air monitoring. The GSTT1 activity in erythrocytes and lymphocyte subpopulations in blood was also measured.**

***Results*—Concentrations of phenanthrene, pyrene, and benzo(a)pyrene in air correlated well with the concentration of total PAHs in air; they could be used for comparisons of different workplaces if the emission compositions were known. The measurement of phenanthrene metabolites in urine proved to be a better biological monitoring variable than the measurement of 1-hydroxypyrene. Significantly more DNA strand breaks in lymphocytes of coke oven workers were found (alkaline filter elution assay); the DNA adduct rate was not significantly increased in workers, but correlated with exposure to PAHs in a semiquantitative manner. The number of sister chromatid exchanges was lower in coke oven workers but this was not significant; thus counting sister chromatid exchanges was not a good variable for biomonitoring of coke oven workers. Also, indications for immunotoxic influences (changes in lymphocyte subpopulations) were found.**

***Conclusions*—The measurement of phenanthrene metabolites in urine seems to be a better biological monitoring variable for exposure to PAHs than measurement of hydroxypyrene. The alkaline filter elution assay proved to be the most sensitive biomarker for genotoxic damage, whereas the postlabelling assay was the only one with some specificity for DNA alterations caused by known compounds.**

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It has been proved by epidemiology that there is an increased risk of cancer for coke oven workers. Various carcinogenic substances can be measured in the workplace atmosphere of coke ovens—for example, benzene, arylamines, and polycyclic aromatic hydrocarbons (PAHs). The increased risk of lung cancer of coke oven workers may be mainly caused by PAHs, important products of pyrolysis at the workplace.[1] There is an urgent need to find sensitive and specific variables for biological monitoring to measure the internal exposure of coke oven workers. These variables may also be used for other workplaces; PAHs are relevant hazards for other groups of workers with exposure to products of pyrolysis—for example, aluminium potroom workers, chimney sweeps, and workers producing graphite electrodes. Also, PAHs are important carcinogenic substances in cigarette smoke. Coke ovens as workplaces have a high exposure to carcinogens, and therefore are suitable for investigating new methods of biological monitoring and biomarkers of effect.

This investigation has been carried out on an older coke plant in Germany to study whether different variables of biological monitoring (excretion of phenanthrene and pyrene metabolites in urine) and biomarkers (measurement of the frequency of DNA single strand breakage (by alkaline filter elution), sister chromatid exchanges, and DNA adducts (by ^{32}P postlabelling)) can be used to assess and classify occupational exposure to the products of pyrolysis and measure genotoxic damage. Also, we measured different lymphocyte subpopulations to measure influences on the cellular immune system.

Materials and methods

SUBJECTS

Twenty nine male coke oven workers in Germany were investigated (16 smokers, 13 non-smokers; 26–56 years old; who had

worked on coke ovens for six to 24 years). We also investigated the same biological effect markers (alkaline filter elution assay, measurement of sister chromatid exchanges, DNA adducts, and lymphocyte subpopulations) in a control group standardised by number, smoking habits, sex, and age (maximum variation of five years within the matched pairs). The controls were healthy and without known exposure to occupational and environmental carcinogens.

Each person answered a questionaire on personal data—namely, smoking and alcohol habits, use of drugs, diseases, holidays during the past weeks, and hobbies—and data on the workplace and working history.

BLOOD AND URINE SAMPLES

Urine was collected from the beginning of the shift in which air monitoring was carried out to the beginning of the shift the next morning.

Venous blood was taken (at the beginning of the morning shift on the day after air monitoring) with 25 ml LH monovettes (Sarstedt, 15 IU lithium heparin/ml blood) and 9 ml monovettes KE (Sarstedt, 1·6 mg EDTA/ml blood). The blood samples were cooled to about 5°C by freeze packs and transported to the laboratory within five hours, with as little vibration as possible. The blood was used immediately. The urine was frozen and stored at −60°C until analysis.

ALKALINE FILTER ELUTION

Heparinised blood was diluted at a ratio of 1:3 (v/v) with Eagle's minimum essential medium in Hank's balanced salt solution and the lymphocytes were isolated on Percoll gradients (Biochrom KG, Seromed). If necessary, any erythrocytes remaining were lysed by incubation with 150 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM Na_2-EDTA for three minutes at 0°C, and the lymphocytes were collected by centrifugation and then resuspended in phosphate buffered saline.

V79 cells (hamster fibroblasts) were cultured in Dulbecco's minimum essential medium with 10% (v/v) horse serum, 10% (v/v) HEPES, and 7·5% sodium hydrogen carbonate and L-glutamine. Confluent growing V79 cells were harvested by trypsination immediately before elution.

Alkaline filter elution was carried out with a slight modification of the method described by Doerjer *et al.*[2]

A suspension containing a million cells was poured on to each filter (polycarbonate filter; pore diameter: 2·0 μM; Nucleopore, Pleasanton, CA, USA). Cells were lysed with 3 ml 2 M NaCl, 10 mM Na_2-EDTA, 0·5% Triton X 100, pH 10 with or without 0·5 mg/ml proteinase K for 60 minutes and then washed with 10 mM Na_2-EDTA, pH 10. The samples were eluted with a solution of 2 M NaCl, 0·02 M Na_2-EDTA, 0·5 M H_3BO_3, and 0·65 M NaOH, pH 12·6. The eluate collected in the first 40 minutes was discarded. The fractions were collected for 10 hours. The eluates, filter extracts, and rinsing fractions of the filter holders and tubes were neutralised with 3 M NaH_2PO_4, 3 M H_3PO_4. After reaction with the fluorochrome Hoechst 33258 (final concentration 0·5 μM) the DNA concentrations were measured at 360 nm excitation and 450 nm emission. Measurements were carried out in triplicate for each subject.

These results were used to calculate the DNA retention (D) of the filter as a percentage of the total amount of DNA; the elution rate (k) was calculated from $k = 2 \log D$. The relative elution rates (k_{rel}) were calculated from the elution rates obtained for the V79 (k_{V79}) cells, which were measured simultaneously ($k_{rel} = k / k_{V79}$).[2-6]

MEASUREMENT OF THE FREQUENCY OF SISTER CHROMATID EXCHANGE

Heparinised blood (400 μl) was incubated for 72 hours at 37°C in 5 ml Roswell Park Memorial Institute (RPMI) 1640 medium (Boehringer) with glutamine, penicillin (115 U/ml), streptomycin (115 μg/ml), amphotericin B (0·29 μg/ml), 20% fetal calf serum (Flow), phytohaemagglutinin M (Gibco), and 10^{-5} M BrdU. Treatment of the cultures with Colcemid and hypotonic KCl solution, fixation, preparation of the chromosomes, and fluorescence plus Giemsa staining of the preparation were carried out according to the method of Perry and Wolff.[7] The frequency of sister chromatid exchange was measured by examining 25 complete second metaphases per subject; the value of sister chromatid exchanges for each subject was taken as the mean of the frequencies of sister chromatid exchange counted per metaphase.

MEASUREMENT OF DNA ADDUCTS

Mononuclear white blood cells were collected after separation from whole blood through a Percoll gradient (Biochrom Seromed, Germany)[6] and stored immediately at −80°C. The DNA was isolated from the cells by treatment with RNase and proteinase K, followed by a solvent extraction procedure.[8] The concentration of DNA was estimated spectrophotometrically and samples were stored at −20°C until analysis.

The DNA adducts were analysed by the nuclease P1 enhancement procedure, essentially as described elsewhere.[9 10] Briefly, 10 μg of DNA was digested to 3'-mononucleotides with 2 μg each of micrococcal nuclease and spleen phosphodiesterase at 37°C overnight. The dephosphorylation of normal nucleotides was carried out at 37°C for one hour by adding 4·8 μg of nuclease P1. Samples were labelled with 100 μCi of commercially prepared (γ-^{32}P)ATP (7000 Ci/mmol; ICN) with 3·5 units of T4 polynucleotide kinase (Boehringer Mannheim, Germany). Purification and resolution of ^{32}P labelled adducts was carried out on PEI cellulose TLC sheets (Macherey and Nagel, Germany) with the following solvent systems: D1, 1 M sodium phosphate, pH 6; D3, 3·5 M lithium formate, 8·5 M urea, pH 3·5; D4, 0·8 M lithium chloride, 8·5 M urea, 0·5 M Tris-HCl, pH 8; D5, 1·7 M sodium phosphate, pH 6. The chromatograms were viewed after autoradiography

Table 1 Concentrations of PAHs (μg/m³) for 27 coke oven workers

PAH	Mean (SD)	Range
Phenanthrene	16·11 (9·00)	4·67–41·91
Anthracene	3·33 (2·63)	0·33–14·08
Fluoroanthene	6·33 (4·32)	1·23–18·94
Pyrene	3·82 (2·54)	0·88–11·31
Benzo(b)naphtho(2,1–d)thiophene	1·01 (0·59)	0·24–2·93
Benzo(ghi)fluoranthene	0·36 (0·27)	0·11–1·26
Benzo(c)PHE	0·44 (0·35)	0·10–1·65
Cyclopenteno(cd)pyrene	0·49 (0·35)	0·08–1·35
Benzo(a)anthracene	3·11 (1·67)	0·92–8·14
Chrysene + triphenylene	3·33 (1·67)	0·97–7·31
Benzofluoranthene(b + j + k)	3·99 (1·87)	1·03–7·74
Benzo(e)pyrene	1·63 (0·76)	0·50–3·20
Benzo(a)pyrene	1·74 (0·82)	0·47–3·55
Perylene	0·41 (0·19)	0·11–0·87
Indeno(1,2,3–cd)pyrene	1·04 (0·45)	0·34–1·93
Dibenz(a,h)anthracene	0·37 (0·18)	0·10–0·88
Benzo(ghi)perylene	1·16 (0·54)	0·41–2·38
Anthanthrene	0·38 (0·30)	0·09–1·06
Coronene	0·16 (0·12)	0·01–0·43
Total PAHs	49·21 (27·42)	13·98–127·37

at −80°C with intensifying screens for at least 72 hours. Adduct levels were measured by Cerenkov counting and calculated taking into account the quantity of DNA labelled and the specific activity of the ATP used. The results are given as the total number of adducts per 10^9 normal nucleotides. A positive control of benzo(a) pyrene-diol-epoxide (BPDE) reacted calf thymus DNA and negative controls of non-adducted calf thymus DNA were routinely assayed with experimental samples. Each sample was analysed at least in triplicate in independent assays.

RELATIVE DISTRIBUTION OF LYMPHOCYTE SUBPOPULATIONS BY FLOW CYTOMETRY

To investigate lymphocyte phenotypic markers 100 μl whole blood of each coke oven worker were incubated with monoclonal antibodies against CD2 (T cells), CD4 (helper/inducer T cells), CD8 (cytotoxic/suppressor T cells), CD19 (B cells), CD56 (NK cells) and CD71 (proliferating cells) (Coulter Electronics, Germany) for 10 minutes at room temperature. After that, blood was lysed, stabilised, and fixed (Q-Prep, Coulter). Measurements were made with a flow cytometer Profile II (Coulter Electronics, Germany).

GSTT1

The phenotype of GSTT1 was measured in lysed erythrocytes. The erythrocytes were collected after the Percoll gradient separation, washed and lysed with the same volume of distilled water. The enzyme assay used has been described by Peter *et al.*[11]

Table 2 Concentrations (mean (SD) in μg/m³) of pyrene, benzo(a)pyrene, and total PAHs for 27 coke oven workers related to smoking and job

Variable (n)	Pyrene	Benzo(a)pyrene	Total PAHs
All (27)	3·82 (2·54)	1·74 (0·82)	49·21 (27·42)
Smokers (14)	3·53 (2·51)	1·62 (0·89)	46·34 (30·16)
Non-smokers (13)	4·13 (2·64)	1·88 (0·75)	52·30 (24·97)
Top side (11)	5·09 (2·23)	2·25 (0·50)	65·13 (17·94)
Coke side (16)	2·95 (2·42)	1·39 (0·83)	38·26 (27·84)
P value (Wilcoxon test)	0·0042	0·0033	0·0013
P value (Jonckheere-Terpstra test)	0·0019	0·0015	0·00067
Top side assistants (9)	5·37 (2·39)	2·25 (0·55)	66·64 (19·54)
Lorry drivers (2)	3·82 (0·04)	2·26 (0·23)	58·32 (7·13)
Coke side assistants (13)	3·25 (2·59)	1·56 (0·82)	42·75 (29·08)
Pusher machine operators (3)	1·64 (0·75)	0·64 (0·25)	18·82 (6·41)
P value (Kruskal-Wallis test)	0·015	0·0069	0·0054
P value (Jonckheere-Terpstra test)	0·00038	0·00025	0·00011

AIR MONITORING

Personal air PAHs were monitored for each coke oven worker during the whole shift with a Dupont P4000 pump, Dupont, USA, and a glass fibre filter (37 mm diameter) impregnated with silicone OV 225 (70 mg). The workers were instructed to put masks on the filter system if they wore masks themselves. This was controlled by one of us who accompanied the workers for the whole shift.

MEASUREMENT OF THE PAHS IN THE AIR SAMPLES

After filter extraction and analytical enrichment the following PAHs were measured: phenanthrene, anthracene, fluoranthene, pyrene, benzo(b)naphtho(2,1-d)thiophene, benzo(ghi)fluoroanthene, benzo(c)phenanthrene, cyclopenteno(cd)pyrene, benz(a)-anthrancene, chrysene+ triphenylene, benzofluoranthenes(b+j+k), benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd) pyrene, dibenz(a,h)anthracene, benzo(ghi)-perylene, anthanthrene, coronene.

Details of the procedure have been described elsewhere.[12] After defrosting to room temperature the urine samples were treated with glucuronidase/sulphatase for 16 hours at 37°C and pH 4·67. Phenols and dihydrodiols were separated by column chromatography on Extrelut with toluene as eluant as described by Grimmer *et al.*[13]

STATISTICAL ANALYSIS

Statistical analysis (Wilcoxon test, Kruskal-Wallis test, Jonckheere-Terpstra test, Pearson correlation) was performed on a personal computer with the SAS program. All biomarker results were examined for correlations with smoking habits, alcohol intake, duration of working on coke ovens, exposure to PAHs, urinary metabolites, and lymphocyte subpopulations.

Results

It was possible to obtain air monitoring data for 27 workers (the personal air samplers did not work in two cases), and to analyse PAH metabolites in 24 hour urine samples from 26 workers (three did not collect urine over the night). Three matched pairs were excluded for biomarker results because the respective workers had worked only for two days after sickness absences of several weeks; we obtained results for the alkaline filter elution assay in 24 workers, counted sister chromatid exchanges in 25 workers and DNA adducts in 23 workers.

PAH CONCENTRATION IN AIR SAMPLES

Table 1 shows the concentrations of PAHs measured by personal air monitoring. There was a high correlation between the concentrations of phenanthrene, pyrene, and benzo-(a)pyrene, and the concentration of total PAHs (r = 0·99, 0·97, and 0·97, Pearson correlation).

Table 2 shows the concentrations of pyrene, benzo(a)pyrene, and total PAHs in relation to smoking habits and jobs at the coke oven.

Table 3 Metabolites (μg/24 h) in the urine in 26 coke oven workers

Metabolite	Mean (SD)	Range
Phenanthrene metabolites:		
Phenols:		
4-OH-phenanthrene	0·02 (0·05)	0–0·18
9-OH-phenanthrene	0 (0·02)	0–0·10
1-OH-phenanthrene	1·54 (1·13)	0·29–4·49
4-OH-phenanthrene	1·70 (1·29)	0·29–4·64
2-OH-phenanthrene	0·82 (0·64)	0·11–2·69
Total phenols	4·07 (2·96)	0·78–11·81
Dihydrodiols*:		
4-OH-phenanthrene	0	0
9-OH-phenanthrene	4·40 (4·47)	0·40–16·37
1-OH-phenanthrene	8·53 (8·56)	0·62–32·26
3-OH-phenanthrene	1·98 (1·52)	0·28–6·42
2-OH-phenanthrene	3·73 (3·70)	0·37–14·09
Total dihydrodiols	18·62 (17·87)	1·95–67·42
Total phenols and dihydrodiols:		
4-OH-phenanthrene	0·02 (0·05)	0–0·18
9-OH-phenanthrene	4·40 (4·48)	0·40–16·37
1-OH-phenanthrene	10·06 (9·54)	0·97–34·65
3-OH-phenanthrene	3·67 (2·61)	0·64–8·88
2-OH-phenanthrene	4·54 (4·25)	0·47–15·90
Total	22·69 (20·43)	2·82–72·92
1-OH-pyrene metabolites:		
Phenol	3·56 (2·02)	0·66–8·71
Dihydrodiol	0·66 (0·54)	0·07–2·26
Total phenol and dihydrodiol	4·22 (2·50)	0·73–10·21

*Measured as phenols after acidic dehydration with acetic acid and sulphuric acid.

Table 4 Excretion of metabolites (mean (SD) of the sums of phenanthrene and pyrene metabolites) by job in 26 workers

	Metabolite excretion (μg/24 h)	
Job (n)	Phenanthrene metabolites	Pyrene metabolites
Top side assistants (8)	35·55 (22·77)	5·86 (2·81)
Lorry drivers (2)	22·44 (11·52)	6·03 (1·32)
Coke side assistants (13)	16·43 (19·38)	3·13 (2·11)
Pusher machine operators (3)	15·66 (9·68)	3·73 (0·30)
P value (Kruskal-Wallis test)	0·085	0·055
P value (Jonckheere-Terpstra test)	0·011	0·015

Table 5 Significant correlations (Pearson) between the 24 h urinary metabolites (mean of the sums of phenanthrene and pyrene metabolites) and the respective air concentrations

Metabolite	r	P value	n*
Phenanthrene in air correlated with:			
9-OH-phenanthrene	0·58	0·0033	24
1-OH-phenanthrene	0·54	0·0069	24
3-OH-phenanthrene	0·55	0·0052	24
2-OH-phenanthrene	0·51	0·010	24
Total phenanthrene metabolites	0·55	0·0049	24
Pyrene in air correlated with:			
1-OH-pyrene	0·44	0·033	24

*There were no air monitoring data in two of 26 workers with metabolite results.

Smokers seemed to be less exposed to PAHs in the workplace in our study. The concentrations of all PAHs were significantly increased on the top of the oven in comparison with the oven side (Wilcoxon test; increase for coronene not significant).

URINARY EXCRETION OF PAH METABOLITES

Table 3 shows the results of the measurement of PAH metabolites and table 4 shows the excretion of metabolites for different jobs.

There were only weak correlations (Pearson) between the metabolites in 24 hour urine samples and the respective air concentrations (table 5).

The influence of smoking on the excretion of the PAH metabolites has been calculated by estimating the content of one cigarette (mainstream smoke) to be 0·3 μg phenanthrene and 0·2 μg pyrene[14] and assuming complete ingestion of these amounts. The mean contribution of smoking to the total uptake was 3% (maximum 11%) for pyrene and 7% (maximum 38%) for phenanthrene. Taking smoking into account improved the correlations between the metabolites in urine and the uptake (from smoking and workplace air) of phenanthrene (Pearson, n = 24, r = 0·71, P = 0·0001) and pyrene (n = 24, r = 0·53, P = 0·0082).

BIOMARKER RESULTS

In the alkaline filter elution assay no difference between the coke oven workers and the control group was found for DNA elution without proteinase K. There was a significant difference with proteinase K, thus indicating significantly more DNA single strand breaks in coke oven workers, but also DNA protein cross links (table 6). Coke oven workers who smoked had higher relative DNA elution rates (with proteinase K) than controls who smoked; this indicated an increased DNA strand breakage frequency in coke oven workers who smoked. We did not find other differences with smoking.

The frequency of sister chromatid exchange was not significantly increased in the controls (table 6). In both groups smokers had more sister chromatid exchanges than non-smokers (coke oven workers P = 0·071; controls P = 0·018).

There were not significantly more DNA adducts in coke oven workers. Coke oven workers who smoked showed more DNA adducts than those who did not smoke (not significant); in the control group the result was opposite.

Table 7 shows the results of the biomarker measurement in coke oven workers with different jobs and PAH exposures. Only DNA adducts were increased (not significant) relative to the exposure to benzo(a)pyrene and total PAHs.

There were some weak correlations between the biomarkers and different factors of possible influence (smoking habits, alcohol intake, GSTT1 activity). There was a correlation between the number of sister chromatid exchanges and alcohol intake (n = 25, r = 0·46, P = 0·021); a correlation with smoking was only found in controls (n = 15, r = 0·63, P = 0·012). We detected a negative correlation between numbers of adducts and GSTT1 activity in erythrocytes of the coke oven workers (n = 20, r = −0·60, P = 0·0056).

We measured lymphocyte subpopulations in the group of coke oven workers and controls. Significantly more NK cells (CD56) and proliferating cells (CD71) were found in coke oven workers than in controls (mean (SD) 19·33 (2·66) *v* 8·13 (2·13) and 30·6 (2·59) *v* 19·91 (2·31), P < 0·01). Contrary to this the numbers of proliferating cells (CD71) in culture after stimulation with PHAs (during counting of sister chromatid exchanges) were higher in controls than in coke oven workers (67·13 (14·82) *v* 54·84 (3·85), P < 0·05)). From all the biomarker results only one positive correlation was found. This was between the number of sister chromatid exchanges in coke oven workers and CD8 positive lymphocytes (T cells; n = 23, r = 0·45, P = 0·031).

We could not find significant correlations between smoking and the biomarker results in

Table 6 Results of the alkaline filter elution, measurements of sister chromatid exchange, adducts in the lymphocytes of coke oven workers and standardised controls

Variable	n	Coke oven workers	Controls	P value (Wilcoxon t test)
Alkaline filter elution:				
Relative DNA elution rates				
(Polycarbonate filter, − proteinase K)	24	0·98 (0·39)	0·95 (0·62)	0·31
Smokers	14	1·05 (0·41)	0·88 (0·68)	0·063
Non-smokers	10	0·88 (0·37)	1·05 (0·54)	0·43
(Polycarbonate filter, + proteinase K)	24	3·08 (2·02)	1·91 (0·79)	0·017
Smokers	14	3·29 (2·23)	1·86 (0·64)	0·0082
Non-smokers	10	2·79 (1·75)	1·98 (0·99)	0·39
Measurements of sister chromatid exchange:				
Sister chromatid exchange:	25	6·16 (0·85)	6·72 (1·55)	0·15
Smokers	15	6·43 (0·92)	7·39 (1·57)	0·093
Non-smokers	10	5·74 (0·54)	5·92 (1·00)	0·91
DNA adduct determination:				
DNA adducts/10^9 nuclei:	23	7·10 (6·59)	4·45 (5·83)	0·16
Smokers	14	7·50 (6·46)	3·86 (1·60)	0·14
Non-smokers	9	6·48 (7·14)	5·83 (7·68)	0·72

Table 7 Biomarker results (mean (SD)) for job and exposure to PAHs

Exposure	Relative DNA elution rate (polycarbonate filter − proteinase K) (n)	Relative DNA elution rate (polycarbonate filter + proteinase K) (n)	Sister chromatid exchange/cell (n)	DNA adducts /10^9 nuclei (n)
Job:				
Top side assistant	0·92 (0·41) (7)	3·26 (1·54) (7)	5·98 (0·98) (8)	9·81 (6·88) (7)
Lorry driver	1·17 (0·18) (2)	2·17 (0·39) (2)	5·28 (0·79) (2)	9·15 (8·27) (2)
Coke side assistant	0·86 (0·36) (12)	2·87 (2·58) (12)	6·25 (0·76) (12)	4·67 (6·08) (11)
Pusher machine operator	1·48 (0·13) (3)	4·08 (0·67) (3)	6·84 (0·41) (3)	8·30 (7·27) (3)
P value (Kruskal-Wallis test)	0·056	0·25	0·17	0·30
P value (Jonckheere-Terpstra test)	0·49	0·87	0·93	0·13
Benzo(a)pyrene (μg/m³):				
< 1	1·17 (0·36) (6)	3·04 (1·24) (6)	6·47 (0·79) (7)	4·77 (6·14) (6)
1–2	0·91 (0·33) (9)	3·13 (2·85) (9)	6·08 (0·63) (9)	6·29 (7·41) (8)
> 2	0·91 (0·46) (9)	3·05 (1·62) (9)	5·99 (1·08) (9)	9·38 (6·10) (9)
P value (Kruskal-Wallis test)	0·40	0·78	0·56	0·27
P value (Jonckheere-Terpstra test)	0·82	0·53	0·84	0·067
Total PAHs (μg/m³):				
< 30	1·03 (0·41) (8)	2·63 (1·32) (8)	6·48 (0·72) (9)	5·14 (5·50) (8)
30–60	0·91 (0·38) (10)	3·54 (2·66) (10)	5·93 (0·80) (9)	7·96 (8·75) (9)
> 60	1·02 (0·44) (6)	3·07 (1·72) (6)	6·02 (1·03) (7)	8·43 (4·11) (6)
P value (Kruskal-Wallis test)	0·94	0·86	0·35	0·48
P value (Jonckheere-Terpstra test)	0·50	0·32	0·85	0·14

the subgroups that smoked. There were no correlations between the biomarker results and exposure to pyrene and phenanthrene when smoking was taken into account.

There were no correlations between the different biomarkers.

Discussion

EXPOSURE TO PAHS

We investigated a group of coke oven workers who were exposed to benzo(a)pyrene concentrations below the German technical exposure limit (5 μg/m³ for coke ovens).[1] Personal air monitoring showed a mean benzo(a)pyrene concentration of 1·74 μg/m³ with a maximum of 3·55 μg/m³ (table 1). We found a good correlation between the phenanthrene, pyrene, and benzo(a)pyrene concentrations in the air and the total PAHs measured. Therefore, all of the three compounds seem to be good indicators of exposure to total PAHs and could be used as indicators in other coke ovens to compare the exposure on different coke ovens, at least if the emission profiles of the coke ovens were known.

Workers on the top of the oven had the highest exposures to PAHs (table 2). The exposure decreased from top side assistants to lorry drivers, workers at the side of the oven and pusher machine operators. Similar results have been reported by other authors.[15 16] The real intake of PAHs may be much higher than indicated by air monitoring. VanRooij *et al*[17] estimated a dermal uptake of benzo(a)pyrene of 51% and of pyrene of 75% in coke oven workers. The intake of benzo(a)pyrene from smoking seems to be low relative to the intake from workplace air: The mean intake of benzo(a)pyrene from smoking (0·04 μg/cigarette, 20 cigarettes/day) may reach 0·8 μg/day, whereas the uptake at the workplace may be about 17 μg/day (1·7 μg/m³, 10 m³ every shift).

BIOLOGICAL MONITORING

We measured different metabolites of phenanthrene and pyrene in 24 hour urine samples (table 3), and found differences of a factor of 20–30 between workers whereas the phenanthrene and pyrene air concentrations differed only by a factor of 15 at the most. This may indicate metabolic differences between people—for example, enzyme polymorphisms. Santella *et al*[18] found differences in excretion of hydroxypyrene of a factor of 20 in people with comparable exposure to PAHs. As in a previous investigation[13] phenanthrene was mainly excreted as dihydrodiol conjugate and pyrene as phenol conjugate.

The sum of both metabolites was greatest in the urine of the workers on the top of the oven (table 4).

Different authors have reported a (significant or at least a semiquantitative) correlation between hydroxypyrene in urine and concen-

trations of pyrene or total PAHs in the workplace air for coke oven and coal liquification workers.[19-21] In our investigation the correlation between concentrations in air and urinary metabolites was better for phenanthrene than for pyrene (table 5). Both metabolites correlated only weakly with the concentration of total PAHs. The correlation between the concentrations of phenanthrene and pyrene and total PAHs in urine was better when smoking was taken into account. So in our investigation the measurement of phenanthrene metabolites seems to be the better variable for biological monitoring. Smoking seems to have important influences on the value of PAH metabolites in urine; as also indicated by other authors.[22-24] Elovaara *et al*[25] concluded that the dermal uptake of pyrene may be very high because in their study on creosote workers an up to 50-fold higher daily urinary excretion of hydroxypyrene was found compared with that calculated from the uptake from air.

BIOMARKERS

Alkaline filter elution showed a significantly increased frequency of DNA single strand breakages in the coke oven workers and additional DNA protein cross links (table 6). There were no significant influences of the workplace, exposure to PAHs, or excretion of metabolites (table 7).

Fuchs *et al*[26] reported that DNA strand breaks in roofers were significantly increased during the working week. It has also been shown in vitro and in vivo that smoking and PAHs may cause DNA single strand breaks[27-32]; PAHs may do this by oxidative damage.[33] It has been shown that the amount of oxidative damage caused by benzo(a)pyrene is 20 times greater than the damage by adducts.[34 35] We also found an increased rate of single strand breaks in patients with oral cancer in whom smoking may have played an important part in carcinogenesis.[36]

We detected increased numbers of DNA adducts in lymphocytes of coke oven workers who smoked, although this was not significant.

DNA adducts did not correlate with exposure to PAHs or metabolite excretion, but increased with semiquantitative increases in exposure to PAHs and benzo(a)pyrene, although again this was not significant (table 7). Most studies on coke oven workers did not find correlations with exposure. Only a few studies have reported correlations between DNA adducts and exposure to PAHs.[21 37-40] Van Schooten *et al*[21] described a good correlation between DNA adducts in workers who smoked and were exposed to PAHs and the excretion of hydroxypyrene in urine.

In a former investigation on a coke oven we found no differences between smokers and non-smokers, in either coke oven workers or controls.[10] The number of adducts was significantly increased in exposed workers, regardless whether smoking was taken into account or not. However, the exposure to benzo(a)pyrene was about twice that in our present study.

In the coke oven workers there was a significantly negative correlation between adducts and GSTT1 activity in erythrocytes. Santella *et al*[41] detected no correlation between the GSTM1 genotype patients with psoriasis treated with coal tar and PAH adducts in white blood cells. Whether GSTT1 metabolises PAHs is not known.[42] Our results may indicate at least an induction of phase II enzymes leading to increased detoxification of DNA reactive metabolites of PAHs.

The frequency of sister chromatid exchange was reduced, but not significantly in coke oven workers and was also lower than that in a larger control group at our institute (n = 78, mean number of sister chromatid exchanges 6·27, not significant). Smokers had more sister chromatid exchanges in both coke oven workers and the control group, but again this was not significant for the coke oven workers. Thus measurement of sister chromatid exchange does not seem to be a good biomarker in coke oven workers; not surprising in view of the conflicting published results of numbers of sister chromatid exchanges in coke oven workers.[16 37 43-45] We also found less sister chromatid exchanges in workers exposed to chromium/nickel and ethylene oxide than in controls.[5 46]

It is known that there are more sister chromatid exchanges in CD8 positive lymphocytes than in CD4 and CD19 positive lymphocytes. Correlations between sister chromatid exchanges and the absolute number of T lymphocytes in blood have been described.[47] The correlation between sister chromatid exchanges and CD8 positive lymphocytes in the blood of coke oven workers might be a consequence of an increased proliferation of CD8 positive lymphocytes,[48] in cultures. This reduced the time to damage chromosomes. In the coke oven workers we found a weak correlation between the number of sister chromatid exchanges and alcohol intake—a similar correlation was found in patients with oral cancer in whom alcohol misuse and smoking were the main causes of carcinogenesis.[49]

In the lymphocyte subpopulations we found significantly increased numbers of NK cells (CD56) and proliferating cells (CD71). This may indicate immunotoxic influences. The reduced number of proliferating cells in culture after stimulation with PHAs might be interpreted as indicating a cellular immune defect due to exposure. Tanigawa *et al*[50] described reduced numbers of CD4 and CD8 positive lymphocytes in the blood of retired chromate workers. Increased numbers of B-cells were found in smokers[51] and reduced numbers in traffic policemen[52] and workers exposed to arylamines.[53] Possible immunotoxic influences of exposure to PAHs need further investigation.

In summary, we investigated a group of coke oven workers exposed to PAHs below the German limit for benzo(a)pyrene in air (5 $\mu g/m^3$ for coke ovens). Concentrations of phenanthrene and pyrene as well as benzo(a)pyrene seem to represent the concentration of total PAHs in air very well; they might be used for comparisons of different workplaces if the emission compositions are

known. Phenanthrene metabolites in urine proved to be better biological monitoring variables than 1-hydroxypyrene, if only the intake of PAHs by inhalation is taken into account. The alkaline filter elution assay showed significantly more DNA strand breaks in lymphocytes of coke oven workers than in controls; the DNA adduct rate was not significantly increased in workers, but correlated with exposure to PAHs in a semiquantitative manner. The measurement of sister chromatid exchanges was not a good variable for biomonitoring of coke oven workers.

Finally, we found indications for immunotoxic influences, which should be studied in further investigations. Detection of genotoxic effects by alkaline filter elution proved to be the most sensitive variable (alkaline filter elution < DNA adducts by postlabelling > sister chromatid exchange). Only DNA adducts in the nuclease P1 modified form of the postlabelling assay showed a relative specificity for PAH adducts. We conclude that in biomarker studies variables with high sensitivity for genotoxic damage and specificity for damage caused by known compounds should always be combined.

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