BIOMARKERS, 1999, VOL. 4, NO. 5, 319–327

Profile of urinary phenanthrene metabolites in smokers and non-smokers

JÜRGEN JACOB*, GERNOT GRIMMER AND GERHARD DETTBARN

Biochemisches Institut für Umweltcarcinogene, Lurup 4, D-22927 Grosshansdorf, Germany

Received 30 November 1998, revised form accepted 25 January 1999

Phenanthrene metabolites (phenols and dihydrodiols) and 1-hydroxypyrene excreted in the 24-h urine of smokers, non-smokers and lung cancer patients, who after heavy smoking became light smokers, were determined and compared. In contrast to 1-hydroxypyrene, no significant differences of the absolute amounts of phenanthrene metabolites were found between smokers and non-smokers. A ratio phenanthrene metabolites/1-hydroxypyrene of 10.4 was observed for non-smokers and 9.9 for lung cancer patients, but 4.2 for smokers. Significantly different ratios for the regiospecific oxidation of phenanthrene were found for smokers when compared with non-smokers (1,2-oxidation vs 3,4-oxidation was 1.45 in the case of smokers, but 2.34 in the case of non-smokers) indicating a cigarette smoke- but not PAH-caused induction of CYP 1A2 in smokers. As a consequence of the degree of PAH exposure the ratio dihydrodiols/phenols depends on the total amount of metabolites excreted. Phenols predominate, equally in smokers and non-smokers after low exposure, while dihydrodiols become more prominent in highly exposed persons (coke plant workers). Both (i) the regiospecific oxidation of PAH and (ii) the ratio of dihydrodiol vs phenol formation may be recognized from the urinary phenanthrene metabolite profile. This pattern mirrors the enzymatic status (balance of the CYP isoforms and epoxide hydrolase) in individuals. Accordingly, more detailed information may be obtained from the urinary metabolite pattern than from 1-hydroxypyrene, commonly used in PAH biomonitoring.

Keywords: biomonitoring, urinary excretion, phenanthrene metabolites, 1-hydroxypyrene, smokers, non-smokers, lung cancer.

Abbreviations: PAH, polycyclic aromatic hydrocarbons; CYP450, cytochrome P450; GC, gas chromatography; MS, mass spectrometry; phenanthrene 1,2-dihydrodiol, 1,2-dihydroxy-1,2-dihydrophenanthrene, phenanthrene 3,4-dihydrodiol, 3,4-dihydroxy-3,4-dihydrophenanthrene; phenanthrene 9,10-dihydrodiol, 9,10-dihydroxy-9,10-dihydrophenanthrene.

Introduction

In most biomonitoring studies of PAH-exposed individuals, 1-hydroxypyrene has been used as a marker metabolite (Jongeneelen *et al.* 1985, 1988; Clonfero *et al.* 1989, Jacob *et al.* 1989, Zhao *et al.* 1990, Angerer *et al.* 1992, van Rooij *et al.* 1993a,b, Vu-Duc and Lafontaine 1996, Mannschreck *et al.* 1996, Kuljukka *et al.* 1997). In rats (Jongeneelen *et al.* 1985) and humans (Zhao *et al.* 1990, Grimmer *et al.* 1994, Kuljukka *et al.* 1997) a correlation between exposure to pyrene, or even to polycyclic aromatic hydrocarbons in general (Jongeneelen *et al.* 1990, Buchet *et al.* 1992, Levin *et al.* 1995, Mannschreck *et al.* 1996, Kuljukka *et al.* 1997) and the urinary excretion of 1-hydroxypyrene has been stated. This correlation might be expected based on the assumption of constant PAH-profiles in which the ratio

* To whom correspondence should be addressed. e-mail: biu.grimmer@t-online.de

Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 1999 Taylor & Francis Ltd
http://www.tandf.co.uk/JNLS/bmk.htm
http://www.taylorandfrancis.com/JNLS/bmk.htm

between pyrene and other PAH does not either vary with time or specifically depend on the kind of emission source. There are, however, also indications that this correlation may depend to a certain extent on individual parameters (Jacob and Grimmer 1996). More detailed information on the metabolic status can be obtained from the profile of urinary phenanthrene metabolites, since (i) this PAH is metabolized at three different molecular regions (1,2-; 3,4- and 9,10-position) significantly depending on the various CYP450 isoforms involved in the oxidation process (Jacob *et al.* 1996) and (ii) in contrast to pyrene, phenanthrene is also converted from the primarily formed arene epoxides by epoxide hydrolase to three different dihydrodiols (Sims 1970, Jacob *et al.* 1982). Since *trans*-dihydrodiols of various PAH (e.g. benzo[*a*]pyrene or dibenzo[*a,l*]pyrene) have been found to operate as proximate carcinogens their formation plays a central role in PAH-induced tumorigenesis.

There are controversial data on whether significant differences between smokers and non-smokers exist regarding the urinary excretion of 1-hydroxypyrene. This may at least partially be due to the fact that the exposure to pyrene by ambient air and by dietary intake is in the same order of magnitude as that caused by cigarette smoking. Elevated 1-hydroxypyrene concentrations in smokers have been reported in some studies (Jongeneelen *et al.* 1990, Sherson *et al.* 1992, van Rooij *et al.* 1994, Levin *et al.* 1995, Angerer *et al.* 1997), whereas in others no such effect could be verified (Jongeneelen *et al.* 1988, Martin *et al.* 1989, Zhao *et al.* 1992, Ny *et al.* 1993).

The goal of the present study was to investigate whether effects of cigarette smoking on the metabolism of PAH exist that can be recognized from the profile of urinary phenanthrene metabolites (phenols and dihydrodiols). To this end, 10 smokers (nine heavy and one occasional smoker) and 10 non-smokers have been investigated over a period of 8 months and their urine collected once every 6 weeks. In addition, the urine of 10 smokers suffering from lung cancer was collected 24 h prior to surgical treatment and all samples analysed for phenanthrene and pyrene metabolites.

Materials and methods

Chemicals

Sephadex LH 20 was purchased from Pharmacia Biotech (D-79111 Freiburg, Germany), β-Glucuronidase/Arylsulphatase ex *Helix pomatia* from Boehringer Mannheim (D-68305 Mannheim, Germany) and other chemicals from Sigma-Aldrich Chemie (D-82041 Deisenhofen, Germany) except 1-hydroxypyrene which was obtained from Promochem GmbH (D-46469-Wesel, Germany). All PAH metabolites (1, 2-, 3-, 4- and 9-hydroxyphenanthrene, phenanthrene-1,2-; 3,4- and -9,10-dihydrodiol) were synthesized as previously described (Jacob *et al.* 1996). Solvents were freshly distilled before use.

Collection of urine

Urine sample were collected during 24 h from 10 non-smoking and 10 smoking probands (nine of which smoked ≥ 25 cigarettes per day and one was an occasional smoker consuming 5–10 cigarettes per week) as well as from 10 smoking lung cancer patients. The samples were deep-frozen and stored at –70 °C until analysed and defrosted prior to clean-up. From the healthy probands, samples were taken every 6 weeks during a total period of 8 months, whereas urine of the patients was sampled only once 24 h prior to surgical treatment. All individuals were fully informed on the objective of the ongoing study. Data were anonymized.

Clean-up of the urine samples

Two parallel subsamples of urine (150 ml each) are buffered to pH 5.67 with sodium acetate (2 N; 1 ml) and acetic acid (2 N; 1 ml). Then 0.3–3 ∝g d_{10}-phenanthrene and d_{12}-chrysene are added as internal

standards. After addition of 100 µl β-glucuronidase/arylsulphatase and 100 ml benzene the sample is shaken for 16 h at 37 °C.

Phenols from the first subsample are collected in the benzene phase which is separated and washed with 10 ml water (pH 5.7). The benzene extract is evaporated to a final volume of 2 ml (rotatory evaporator, 65 mbar, 35 °C, pressure equilibration under nitrogen).

For the determination of the original phenols the extract from the first subsample is methylated, while the second subsample is acidified with 2 N KCl and 2 N hydrochloric acid to give a final pH of 2.0 and shaken for 16 h at 37 °C to convert dihydrodiols into phenols.

For the determination of the dihydrodiols (as the difference between the total phenols obtained after acidic treatment and the original phenols) the acidified sample is treated as above.

The two different subsamples containing now phenols exclusively ([i] original phenols and [ii] phenols formed from the dihydrodiols by acid-catalysed water elimination plus original phenols) are then derivatized to the corresponding methyl ethers by treatment with diazomethane. To this end, 2 ml of methanol and 20 ml of diazomethane in ether [20 g *N*-methyl-*N*-nitroso-4-toluenesulphonamide is dissolved in 100 ml ether, cautiously added to an aqueous/methanolic solution of potassium hydroxide under stirring (50 ml, 15 % KOH, 40 °C) and the diazomethane solution in ether distilled] are added to the phenols-containing benzene solution. After 3 h at room temperature, the mixture is evaporated to 1 ml.

The residue is distributed between cyclohexane (20 ml), benzene (10 ml) and water (20 ml) and the cyclohexane/benzene phase washed with another 20 ml of water. The cyclohexane/benzene phase is evaporated to 1 ml and filtered through silica (1.5 g; 13 % water content) by elution with 80 ml cyclohexane. The solvent is evaporated to a volume of 1 ml to which 2 ml of propanol-2 are added to remove the remaining cyclohexane by further evaporation. The residue is purified by LH 20 Sephadex chromatography (5 g) with propanol-2 as eluent (20 ml h^{-1}). The fraction 18–80 ml contains the methoxyphenanthrenes and 1-methoxypyrene.

Gas chromatography/mass spectrometry (GC/MS)

The GC separation of the methoxy derivatives is carried out using a Hewlett Packard (HP) 5890 Series II instrument equipped with a split/splitless injector and a PTE 5 Supelco 30 m × 0.25 mm column (0.2 ∝m film thickness). An HP-MSD 5972 mass detector is used as detector and an HP-chemstation-MS as integrator. The sample (1 µl) is injected at 250 °C. The following temperature programme results in an optimum resolution of the peaks; (1) 75 °C to 140 °C with 15 °C min^{-1}; (2) 140 °C to 175 °C with 2 °C min^{-1} (3) 175 °C to 280 °C with 10 °C min^{-1}. The split is opened 1.5 min after injection.

For the quantitation, the molecular ion (M^+) and the fragments (M–15) and (M–43) are used relating them to the integral of the molecular ion of the internal standards (d_{10}-phenanthrene and d_{12}-chrysene) after calibration. Further details of the method (reproducibility, variation coefficients, standard deviation, quantitation of the acid-catalysed water elimination from dihydrodiols) have been published elsewhere (Grimmer *et al.* 1997b).

Results and Discussion

Mean and standard deviations of the absolute amounts of phenanthrene phenols and dihydrodiols as well as those of 1-hydroxypyrene excreted in the urine of 10 male smokers (nine smoker and one occasional smoker) and 10 male non-smokers during 24 h over a period of 8 months (one measurement every 6 weeks) are presented in table 1. In the table also the ratio (i) 1,2- vs 3,4-oxidation products of phenanthrene, (ii) phenanthrene dihydrodiols vs phenanthrene phenols and (iii) total phenanthrene metabolites vs 1-hydroxypyrene are shown.

The table also compiles data obtained from urine samples of lung cancer patients (all initially were heavy smokers [20–40 cigarettes per day] who then became light smokers [3–6 cigarettes per day]) collected 24 h prior to surgical treatment.

The amounts of urinary phenanthrene metabolites do not significantly depend on smoking. Although slightly higher concentrations were found in the case of smokers (means of 10 smokers vs 10 non-smokers were 2528 ± 1074/2270 ± 1242 ng total phenanthrene metabolites/24-h urine) the variation was comparably large for both groups. For the lung cancer patients the variation was even greater (2861 ±

Table 1. Phenanthrene metabolites and 1-hydroxypyrene excreted in the urine (ng per 24 h) by smokers and non-smokers measured at six time-points over a period of 8 months and cancer patients[a]) (means and standard deviations).

	Smokers $n=9$, $m=54$[b])		Occasional smoker $n=1$, $m=6$[b])		Non-smokers $n=10$, $m=60$[b]		Cancer patients[a] $n=10$, $m=10$[b]	
	MW	SD	MW	SD	MW	SD	MW	SD
Phenanthrene phenols (total)	1654	± 665	1817	± 836	1422	± 739	1492	±1071
1-OH	412	± 179	761	± 418	542	± 344	380	± 301
2-OH	315	± 160	411	± 197	284	± 155	387	± 284
3-OH	598	± 267	421	± 218	395	± 187	596	± 391
4-OH	97	± 52	75	± 40	90	± 118	55	±67
9-OH	232	±112	149	±92	111	± 52	74	± 76
Phenanthrene dihydrodiols (total)	874	± 635	2052	± 929	855	± 720	1368	± 1185
1,2-Diol	585	± 431	1693	± 794	665	± 555	1129	± 1019
3,4-Diol	222	± 203	330	± 255	148	± 173	244	± 299
9,10-Diol	74	± 73	29	± 11	42	± 53	63	±58
Phenanthrene metabolites (total)	2428	± 1074	3869	± 1384	2277	±1242	2861	± 2097
Regiospecific oxidation (%)								
1,2-Oxidation	51	± 6	73	± 6	65	±5	63	± 8
3,4-Oxidation	37	± 5	22	± 4	28	± 4	31	± 7
9,10-Oxidation	12	± 3	5	± 2	7	± 3	5	± 4
Ratio: 1,2/3,4-oxidation	1.45	± 0.39	3.3	± 0.8	2.34	± 0.53	2.17	± 0.66
Ratio: dihydrodiols/phenols	0.53	± 0.44	1.26	± 0.52	0.62	± 0.48	0.92	± 0.55
1-OH-pyrene[c]	603	± 483	216	± 115	218	± 159	290	± 238
Ratio Phenanthrene metabolites/1-OH-pyrene	4.2		17.9		10.4		9.9	

[a] Heavy smokers who smoked, however, only 3–10 cigarettes during the day prior to sampling.
[b] n = number of individual probands, m = number of measurements.
[c] According to t-test significant differences between smokers and non-smokers ($P = 0.000001$).

2097 ng/24-h urine) and no correlation was found either to the long-term cigarette consumption (e.g. 10 or 30–40 cigarettes per day) or to the number of cigarettes smoked during the previous 24 h.

Based on an average of about 10 ng m^{-3} phenanthrene and 3 ng m^{-3} pyrene in ambient air (Grimmer *et al.* 1997a) and a daily respiratory volume of 11 m^3 (Jacob and Grimmer 1996) the phenanthrene and pyrene masses inhaled may be calculated to be 110 ng phenanthrene and 33 ng pyrene. A daily consumption of 20 cigarettes for smokers (phenanthrene/pyrene masses per cigarette: 85 ng/65 ng [Lee *et al.* 1976]) results in 1700 ng phenanthrene and 1300 ng pyrene, which is about 10–40 times the amount inhaled by breathing. Since no significant increase of phenanthrene metabolites was found for smokers, it is reasonable to assume that the metabolic pathway leading to the urinary excretion of the analysed phenanthrene metabolites is saturated. In the case of pyrene, however, significantly higher amounts of urinary 1-hydroxypyrene were measured for smokers (means: 603 ± 483 ng/24h urine for smokers, but 218 ± 157 ng/24 h urine for non-smokers; i.e. a ratio of 2.8) supporting previously published studies (Jongeneelen *et al.* 1989, Sherson *et al.* 1990, 1992, Grannela and Clonfero 1993, van Rooij *et al.* 1994, Levin *et al.* 1995, Angerer *et al.* 1997, Sithisarankul *et al.* 1997). Accordingly, the mean of the ratio phenanthrene metabolites/l-hydroxpyrene is 4.2 in smokers and about 10.4 in non-smokers (factor 2.5), reflecting the higher intake of pyrene by smokers. The

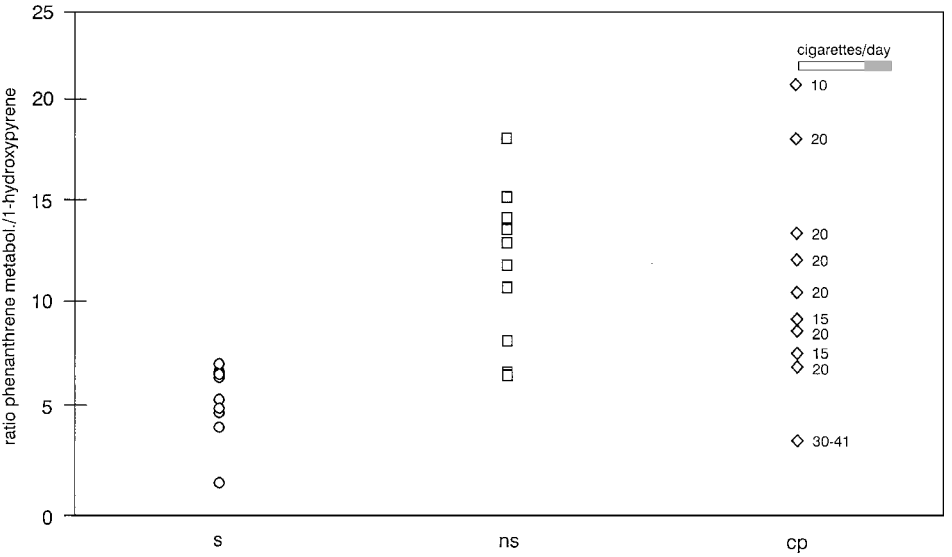

Figure 1. Ratio of phenanthrene metabolites/1-hydroxypyrene in the urine of smokers (s), non-smokers (ns) and smoking lung cancer patients (cp). The latter became light smokers (3–6 cigarettes per day) after long period of heavy smoking (20–40 cigarettes per day).

ratio phenanthrene metabolites/l-hydroxypyrene found for the lung cancer patients (9.9) who after heavy smoking (20–40 cigarettes per day) became light smokers (3–6 cigarettes per day) resembles that of non-smokers rather than that of smokers (figure 1).

As shown in figure 2 a significant difference between smokers and non-smoker was found for the regiospecific oxidation of phenanthrene. The ratio 1,2-oxidation vs 3,4-oxidation was 1.2–2.1 for smokers (mean: 1.45). One subject (no. 45) showed a ratio of 3.3 However, this individual was an occasional smoker of 5–10 cigarettes per *week*, whereas the others smoked ≥ 25 cigarettes per *day*. A significantly higher ratio was measured for non-smokers (1.9–2.9; mean: 2.3). A similar mean ratio of 2.2 was found for the lung cancer patients, although most of them smoked between three and six cigarettes during the previous day. The ratio of the regiospecific oxidation of phenanthrene (1,2-oxidation vs 3,4-oxidation) in highly PAH-exposed coke plant workers (mean: 2.7 (1.5–3.7), data taken from Grimmer *et al.* (1993)), on the other hand, resembles that found in non-smokers (mean: 2.3), indicating that PAH induce a different CYP 450 isoform than cigarette smoke does, probably CYP 1A1.

These data suggest that the 3,4-oxidation of phenanthrene becomes more prominent in smokers which may be explained by an induction of the CYP450 1A2 system in the liver. Induction of human liver microsomal CYP 1A2 by cigarette smoke has been repeatedly reported (Pelkonen *et al.* 1986, Sesardic *et al.* 1987, 1990, Sherson *et al.* 1992, Sithisarankul *et al.* 1997). It has been shown with genetically engineered V79 Chinese hamster cells specifically expressing various CYP isoforms that the ratio of phenanthrene 1,2-oxidation vs 3,4-oxidation is 14.1 for human CYP 1A1 but only 1.1 for human CYP 1A2 (Jacob *et al.* 1996), indicating that the 3,4-oxidation plays a more important role in the case of CYP 1A2. The

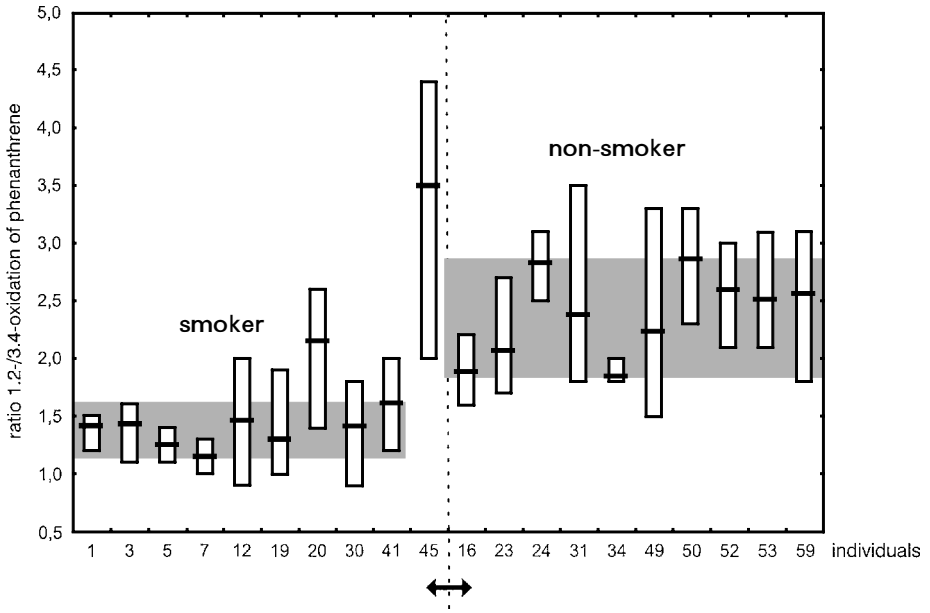

Figure 2. Ratio of the 1,2-/3,4-oxidation products of phenanthrene in the urine of smokers and non-smokers.

same ratio, however, is also found with human CYP 3A4 (Jacob *et al.* 1996) so that the induction of this form cannot be ruled out.

No differences between smokers and non-smokers were found for the ratio of urinary dihydrodiols/phenols (smokers: 0.53; non-smokers: 0.62) nor was the ratio markedly different in the case of the lung cancer patients (0.92), i.e. phenols were the main metabolites excreted by all groups. In contrast to this, persons with high PAH exposure, such as coke plant workers exhibited a mean ratio of about 5 (Grimmer *et al.* 1993). In addition, a correlation ($r = 0.813$) between this ratio and the total phenanthrene metabolites was observed (figure 3). These data suggest that the conversion of primarily formed epoxides into dihydrodiols is more efficient under these conditions – possibly a consequence of PAH-dependent induction of the epoxide hydrolase.

Conclusions

Comparing smokers and non smokers, no significant differences of the absolute amounts of phenanthrene metabolites were found. This may indicate that the metabolic pathway leading to the urinary excretion of the analysed phenanthrene metabolites is saturated and that further pathways may be involved in the excretion of phenanthrene. In contrast to this, 1-hydroxypyrene excretion was increased by a factor of 2.8 in smokers, suggesting that smoking significantly contributes to it.

A phenanthrene metabolites/l-hydroxypyrene ratio of 10.4 was found for non-smokers and a similar value of 9.9 for lung cancer patients who became light smokers shortly before urine samples had been taken for this study, while the ratio was 4.2 for smokers.

A characteristic ratio of the regiospecific phenanthrene oxidation products

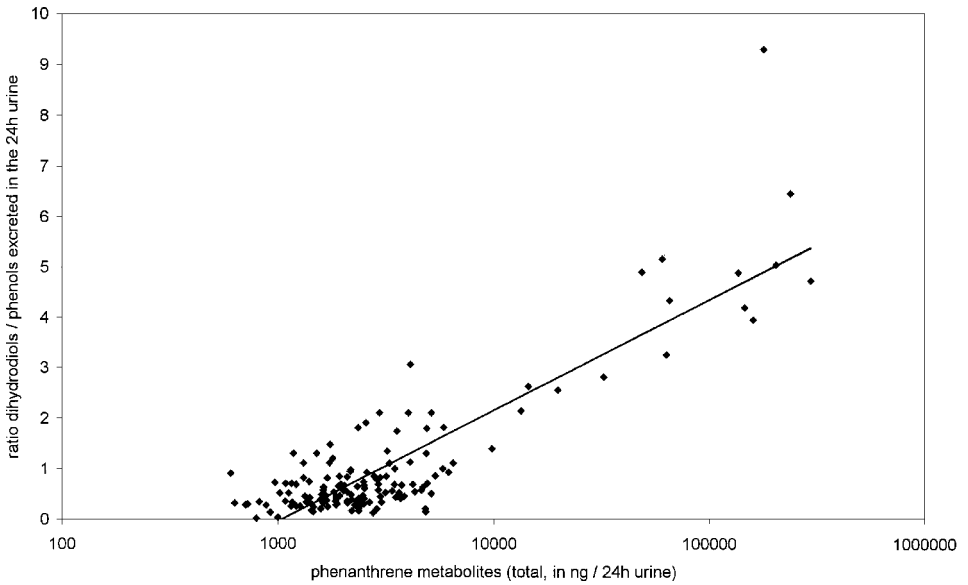

Figure 3. Ratio of phenanthrene dihydrodiols/phenols in dependence on the total phenanthrene metabolites excreted in the urine.

(1,2-oxidation vs 3,4-oxidation) of 1.45 was observed in smokers compared with 2.3 in the case of non-smokers and 2.2 in the case of lightly smoking lung cancer patients. The increase of the 3,4-oxidation of phenanthrene indicates an induction of the CYP 1A2 (or CYP 3A4) monooxygenase system in smokers obviously caused by cigarette smoke constituents other than PAH, since in the urine of heavily PAH-exposed coke plant workers a ratio of 2.6 has been observed (as calculated from Grimmer *et al.* 1993) which resembles that found in non-smoker. From animal experiment (nicotine-pretreated rats) it appears to be likely also that nicotine can be ruled out to be responsible for this effect since this alkaloid did not cause qualitative or quantitative changes of rat liver cytochromes P450 (Schmoldt *et al.* 1988).

The ratio of urinary dihydrodiols vs phenols was found to depend on the total amount of metabolites and, hence, on the degree of PAH-exposure. Phenols predominate (ratio dihydrodiols/phenols $''$ 1.0), equally in smokers and non-smokers after low PAH-exposure (1–10 $\propto$g day^{-1}), while dihydrodiols become more prominent (ratio dihydrodiols/phenols > 2) in highly exposed persons such as coke plant workers (1–10 mg day^{-1}). These data suggest that, apart from the CYP 1A1, the epoxide hydrolase is also induced by high PAH exposure. The PAH concentration of cigarette smoke is obviously too low to result in such an effect though its carcinogenic potency is unquestionable (Grimmer *et al.* 1988).

In conclusion, the analysis of urinary phenanthrene yields information on the balance of the PAH-metabolizing cytochrome isoforms and the epoxide hydrolase activity in individuals which may not be obtained by determining 1-hydroxypyrene in the urine exclusively. It would also not have been possible to recognize the cigarette smoking-caused induction of CYP 1A2 by analysing the urinary excretion of 1-hydroxypyrene since pyrene is a very poor substrate for this cytochrome isoform (Jacob *et al.*, unpublished data).

Acknowledgements

The authors wish to express their gratitude to PD Dr G. Scherer, ABF München and Dr P. C. Bauer, Abt.Pneumologie/Allergologie d. Ruhrland Klinik Essen, for supplying them with urine sample of smokers and non-smoker as well as of lung cancer patient, respectively. They are also greatly indebted to PD Dr G. Scherer and Dr A. Seidel, Mainz for constructive discussions.

References

ANGERER, J., SCHALLER, K. H. and LEHNERT, G. 1992, Bio-Monitoring von beruflichen Belastungen gegenüber polycyclischen aromatischen Kohlwenwasserstoffen – Hochdruckflüssigkeitschromatographische Bestimmung des Metaboliten 1-Hydroxypyren im Harn, 4.Intern.Koll. p. 12, Straβburg 22.–23.10.1992.

ANGERER, J., GÜNDEL, J., MANNSCHRECK, C., EWERS, U. and BÜTTNER, K. 1997, Beurteilung der PAH-Belastung Anwohner eines Industriegebiets in der Bundesrepublik Deutschland. Bestimmung von 1-Hydroxypyren, 1-, 12+ 9-, 3- und 4-Hydroxyphenanthren im Harn. *Umweltmedizin und Forschung und Praxis*, **2**, 17–22.

BUCHET, J. P., GENNART, J. P., MERCADO-CALDERON, F., DELAVIGNETTE, J. P., CUPERS, L. and LAUWERYS, R. 1992, Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of the urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. *British Journal of Industrial Medicine*, **49**, 761–768.

CLONFERO, E., ZORDAN, M., VENIER, P., PALEOLOGO, M., LEVIS, A. G., COTTICA, D., POZZOLI, L., JONGENEELEN, F. J., BOS, R. P. and ANZION, R. B. M. 1989, Biological monitoring of human exposure to coal tar. Urinary excretion of total PAH, 1-hydroxypyrene and mutagens in psoriatic patient. *International Archives of Occupational and Environmental Health*, **61**, 363–369.

GRANNELA, M. and CLONFERO, E. 1993, Urinary excretion of 1-pyrenol in automotive repair workers. *International Archives of Occupational and Environmental Health,* **65**, 241–245.

GRIMMER, G., BRUNE, H., DETTBARN, G., NAUJACK, K.-W., MOHR, U. and WENZEL-HARTUNG, R. 1988, Contribution of polycyclic aromatic compounds to the carcinogenicity of sidestream smoke of cigarettes evaluated by implantation into the lungs of rats. *Cancer Letters*, **32**, 173–177.

GRIMMER, G., DETTBARN, G. and JACOB, J. 1993, Biomonitoring of polycyclic aromatic hydrocarbons in highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites (phenols and dihydrodiols). *International Archives of Occupational and Environmental Health*, **65**, 189–199.

GRIMMER, G., DETTBARN, G., NAUJACK, K.-W. and JACOB, J. 1994. Relationship between inhaled PAH and urinary excretion of phenanthrene, pyrene and benzo[*a*]pyrene metabolites in coke plant workers. *Polycyclic Aromatic Compounds* **5**, 269–277.

GRIMMER, G., JACOB, J. and NAUJACK, K.-W. 1997a, Atmospheric emission of polycyclic aromatic hydrocarbons in sampling areas of the German Environmental Specimen Bank. Method for the precise measurement of gaseous and particle-associated polycyclic aromatic hydrocarbons in the sub-nanogram range using deuterated internal standards. *Chemosphere*, **24**, 2213–2226.

GRIMMER, G., JACOB, J., DETTBARN, G. and NAUJACK, K.-W. 1997b, Determination of urinary metabolites of polycyclic aromatic hydrocarbons (PAH) for the risk assessment of PAH-exposed workers. *International Archives of Occupational and Environmental Health*, **69**, 231–239.

JACOB, J. and GRIMMER, G. 1996, Metabolism and excretion of polycyclic aromatic hydrocarbons in rat and in human. *Central European Journal of Public Health*, **4** (Suppl.), 33–39.

JACOB, J., SCHMOLDT, A. and GRIMMER, G. 1982, Influence of monoxygenase inducers on the metabolite profile of phenanthrene in rat-liver microsomes. *Toxicology*, **25**, 333–343.

JACOB, J., BRUNE, H., DETTBARN, G., GRIMMER, G., HEINRICH, U., MOHTASHAMIPUR, E., NORPOTH, K., POTT, F. and WENZEL-HARTUNG, R. 1989, Urinary and faecal excretion of pyrene and hydroxypyrene by rats after oral, intraperitoneal, intratracheal or intrapulmonary application. *Cancer Letters*, **46**, 15–20.

JACOB, J., RAAB, G., SOBALLA, V., SCHMALIX, W. A., GRIMMER, G., GREIM, H., DOEHMER, J. and SEIDEL, A. 1996, Cytochrome P450-mediated activation of phenanthrene in genetically engineered V79 Chinese hamster cells. *Environmental Toxicology and Pharmacology*, **1**, 1–11.

JONGENEELEN, F. J., ANZION, R. B. M., LEIJDEKKERS, CH.-M., BOS, R. P. and HENDERSON, P. TH. 1985, 1-Hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product. *International Archives of Occupational and Environmental Health*, **57**, 47–55.

JONGENEELEN, F. J., ANZION, R. B. M., SCHEEPERS, P. T. J., BOS, R. P., HENDERSON, P. T., NIJENHUIS, E. H., VEENSTRA, S. J., BROUNS, R. M. E. and WINKES, A. 1988, 1-Hydroxypyrene in urine as a biological indicator of exposure to polycyclic aromatic hydrocarbons in several work environments. *Annals of Occupational Hygiene*, **32**, 35–43.

JONGENEELEN, F. J., ANZION, R. B. M., THEUWS, J. L. G. and BOS, R. P. 1989, Urinary 1-

hydroxypyrene levels in workers handling petroleum coke. *Journal of Toxicology and Environmental Health*, **26**, 133–136.

JONGENEELEN, F. J., VAN LEEUWEN, F. E., OOSTERINK, S., ANZION, R. B. M., VAN DER LOOP, F., BOS, R. P. and VAN VEEN, H. G. 1990, Ambient and biological monitoring of coke oven workers; determinants of the internal dose of polycyclic aromatic hydrocarbons. *British Journal of Industrial Medicine*, **47**, 454–461.

KULJUKKA, T., VAARANRINTA, R., MUTANEN, P., VEIDEBAUM, T., SORSA, M., KALLIOKOSKI, P. and PELTONEN, K. 1997, assessment of occupational exposure to PAHs in an Estonian coke oven plant – correlation of total external exposure to internal dose measured as 1-hydroxypyrene concentration. *Biomarkers*, **2**, 87–94.

LEE, M. L., NOVOTNY, M. and BARTLE, K. D. 1976, Gas chromatography/mass spectrometric and nuclear magnetic resonance spectrometric studies of carcinogenic polynuclear aromatic hydrocarbons in tobacco and marijuana smoke condensates. *Analytical Chemistry*, **48**, 405–416.

LEVIN, J. O., RHÉNAND, M. and SIKSTRÖM, E. 1995, Occupational PAH exposure: urinary 1-hydroxypyrene levels of coke oven workers, aluminium smelter pot-room workers, road pavers, and occupationally non-exposed persons in Sweden. *Science of the Total Environment*, **163**, 169–177.

MANNSCHRECK, C., GÜNDEL, J. and ANGERER, J. 1996, Occupational exposure to PAHs – Biological monitoring of hydroxylated metabolites. *Polycyclic Aromatic Compounds*, **11**, 11–18.

MARTIN, F., HOEPFNER, I., SCHERER, G., ADLKOFER, F., DETTBARN, G. and GRIMMER, G. 1989, Urinary excretion of hydroxy-phenanthrenes after intake of polycyclic aromatic hydrocarbons. *Environment International*, **15**, 41–47.

NY, E. T., HEEDERIK, D., KROMHOUT, H. and JONGENEELEN, F. J. 1993, The relationship between polycyclic aromatic hydrocarbons in air and in urine of workers in a Söderberg potroom. *American Industrial Hygiene Association Journal*, **54**, 277–284.

PELKONEN, O., PASANEN, M., KUHA, H., GACHALYI, B., KAIRALUOMA, M., SONTANIEMI, E. A., PARK, S. S., FRIEDMAN, F. K. and GELBOIN, H. V. 1986, The effect of cigarette smoking on 7-ethoxyresorufin O-deethylase and other monooxygenase activities in human liver: analysis with monoclonal antibodies. *British Journal of Clinical Pharmacology*, **22**, 125–134.

SCHMOLDT, A., JACOB, J. and GRIMMER, G. 1988, Metabolism of benz[*a*]anthracene catalyzed by liver microsomes of untreated and nicotine-pretreated rats. *Klin. Wochenschr.,* **66** (Suppl. XI), 108–113.

SESARDIC, D., DAVIES, D. S., EDWARDS, R. and BOOBIS, A. R. 1987, Identification of the major isoenzyme of cytochrome P450 induced by cigarette smoking in man. In *Enzyme Induction in Man* e. E. A. Sotaniemi and O. Pelkonen, eds (London, New York, Philadelphia: Taylor and Francis), pp. 33–46.

SESARDIC, D., PASANEN, M., PELKONEN, O. and BOOBIS, A. R. 1990, Differential expression and regulation of members of the cytochrome P450 IA gene subfamily in human tissues. *Carcinogenesis*, **11**, 1183–1188.

SHERSON, D., SABRO, P., SIGSGAARD, T., JOHANSEN, F. and AUTRUP, H. 1990, Biological monitoring of foundry workers exposed to polycyclic aromatic hydrocarbons. *British Journal of Industrial Medicine*, **47**, 448–453.

SHERSON, D., SIGSGAARD, T., OVERGAARD, E., LOFT, S., POULSEN, H. and JONGENEELEN, F. J. 1992, Interaction of smoking, uptake of polycyclic aromatic hydrocarbons, and cytochrome P450 IA2 activity among foundry workers. *British Journal of Industrial Medicine*, **49**, 197–202.

SIMS, P. 1970, Qualitative and quantitative studies on the metabolism of a series of aromatic hydrocarbons by rat-liver preparations. *Biochemical Pharmacology*, **19**, 795–818.

SITHISARANKUL, P., VINEIS, P., KANG, D., ROTHMAN, N., CAPORASO, N. and STRICKLAND, P. 1997, Association of 1-hydroxypyrene-glucuronide in human urine with cigarette smoking and broiled or roasted meat consumption. *Biomarkers*, **2**, 217–221.

VAN ROOIJ, J. G. M., BODELIER-BADE, M. M. and JONGENEELEN, F. J. 1993a, Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. *British Journal of Industrial Medicine*, **50**, 623–632.

VAN ROOIJ, J. G. M., VAN LIESHOUT, E. M. A., BODELIER-BADE, M. M. and JONGENEELEN, F. J. 1993b, Effect of the reduction of skin contamination on the internal dose of creosote workers exposed to polycyclic aromatic hydrocarbons. *Scandinavian Journal of Work, Environment and Health*, **19**, 200–207.

VAN ROOIJ, J. G. M. VEEGER, M. M. S., BODELIER-BADE, M. M., SCHEEPERS, P. T. J. and JONGENEELEN, F. J., 1994 Smoking and dietary intake of polycyclic aromatic hydrocarbons as a source of interindividual variability in the baseline excretion of 1-hydroxypyrene in urine. *International Archives of Occupational and Environmental Health*, **66**, 55–65.

ZHAO, Z.-H., QAN, W.-Y., and TIAN, D.-H. 1990, Urinary 1-hydroxypyrene as an indicator of human exposure to ambient polycyclic aromatic hydrocarbons in a coal burning environment. *Science of the Total Environment*, **92**, 145–154.

ZHAO, Z.-H., QAN, W.-Y. and TIAN, D.-H. 1992, Experiments on the effects of several factors on the 1-hydroxypyrene level in human urine as an indicator of exposure to PAH. *Science of the Total Environment*, **113**, 197–207.