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

Terhi Kuljukka, Raija Vaaranrinta, Pertti Mutanen, Toomas Veidebaum, Marja Sorsa, Pentti Kalliokoski and Kimmo Peltonen

The exposure of cokery workers to polynuclear aromatic hydrocarbons at an Estonian oil shale processing plant was assessed by using occupational hygiene and biomonitoring measurements which were carried out twice, in midwinter and in the autumn. To assess the external dose of polynuclear aromatic hydrocarbons, pyrene and benzo[*a*]pyrene concentrations were measured from the breathing zone of workers during a workshift. Skin contamination with pyrene and benzo[*a*]pyrene was assessed by skin wipe sampling before and after the workshift. As a biomarker of overall exposure to polynuclear aromatic hydrocarbons, and as an integral of all absorption routes of pyrene, 1-hydroxypyrene concentration was measured from post shift urine samples. Of the personal air samples, 18% exceeded the Finnish threshold limit value of benzo[*a*]pyrene (10 μg m^{-3}). Mean value (two separate measurements together) for benzo[*a*]pyrene was 5.7 μg m^{-3} and for pyrene, 8.1 μg m^{-3}. Based on skin wipe sample analyses, the skin contamination was also obvious. The mean value of benzo[*a*]pyrene in the samples collected after the shift was 1.2 ng cm^{-2}. Benzo[*a*]pyrene was not found in control samples. The mean value of urinary 1-hydroxypyrene concentration was 6.0 μmol mol^{-1} creatinine for the exposed workers and 0.5 μmol mol^{-1} creatinine for the controls. This study undoubtedly shows the usefulness of 1-hydroxypyrene as an indicator of internal dose of polynuclear aromatic hydrocarbons. It can be concluded that the cokery workers at the Kohtla-Järve plant are exposed to high concentrations of polynuclear aromatic compounds, and the exposure level is considerably higher during the winter measurements.

Keywords: *polynuclear aromatic hydrocarbons, cokery work, industrial hygiene, biomonitoring, 1-hydroxypyrene.*

Abbreviations: *B[a]P, benzo[a]pyrene; 1-OHP, 1-hydroxypyrene; PAH, polycyclic aromatic hydrocarbon.*

Terhi Kuljukka, Raija Vaaranrinta, Kimmo Peltonen *(author for correspondence (Molecular Dosimetry Group) and* **Pertti Mutanen** *(Department of Epidemiology and Biostatistics) are at the Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, SF-00250, Helsinki, Finland;* **Toomas Veidebaum** *is at the Institute of Experimental and Clinical Medicine, Tallinn, Estonia;* **Marja Sorsa** *is at the Ministry of Education, Helsinki, Finland; and* **Pentti Kalliokoski** *is at the University of Kuopio, Department of Environmental Sciences, Kuopio, Finland.*

Introduction

Oil shale processing, in specially designed retorts, for energy supply and further refining to a variety of raw materials for petrochemistry is an important field of industry and the export trade in Estonia. In Estonia about 99% of the electric energy production is based on oil shale (Jürgenfeld *et al.* 1994). The first industrial shale oil factory in Kohtla-Järve started in 1924 while the production of oil coke began in 1964 (Rooks 1994). Coke production at the Kohtla-Järve oil shale plant gets its raw material partly from the retorting process of oil shale and the rest is imported from Russia. The heaviest bitumenous fractions from the retorting process are led into the coke ovens. On average 50 000 tons of raw material is burnt annually (Dr Veidebaum). Production of oil coke differs from the traditional cokery process in that the raw material is in a liquid state compared with the solid coal normally used, and the chemical composition is also unique. These differences might affect the chemical reactions which happen during the burning process and by means of which compounds are released into the ambient environment.

In the cokery process coal is destructively distilled into coke. The workers are exposed to a complex mixture of gaseous, liquid, and, in particular, particulate emissions. The exposure of coke oven workers to high concentrations of polycyclic aromatic hydrocarbons (PAHs) has evoked especial concern, mainly because in epidemiological studies many of them have been shown to be carcinogenic. There is considerable evidence that exposure to occupational emissions causes elevated risk of cancer of the skin and lung among cokery workers (IARC 1984, 1987).

In recent coke oven studies reported B[*a*]P concentrations have varied from 2.7 μg m^{-3} to 69 μg m^{-3}, and pyrene concentrations from 35 μg m^{-3} to 200 μg m^{-3} (Bjørseth *et al.* 1978, Yrjänheikki *et al.* 1994). In a Swedish cokery the concentration of 14 PAH compounds was determined to be 6–570 μg m^{-3} (Reuterwall *et al.* 1991).

Evaluation of human exposure to PAHs by carrying out only environmental monitoring may be inadequate due to the strong adsorption of some PAH compounds onto the surface of particulates (Dufresne *et al.* 1987, Lesage *et al.* 1987) and the significance of skin absorption (Jongeneelen *et al.* 1988a, VanRooij *et al.* 1992, 1993a, Elovaara *et al.* 1995). Furthermore, there is a need for a reliable and comprehensive measure of internal dose for risk assessment. In several occupational hygiene studies 1-hydroxypyrene has been shown to be a sound marker for PAH exposure, and to correlate well with total PAH exposure from multiple sources (Jongeneelen *et al.* 1990, Zhao *et al.* 1990, Buchet *et al.* 1992). Despite the numerous supporting observations for the use of 1-OHP as a measure of internal pyrene and total PAHs many things also affect the interpretation of exposure data. An important source of PAH exposure and the main confounding factor in environmental or occupational PAH exposure measurements is smoking. It is a well known fact that tobacco smoke contains numerous PAH compounds. There is approximately 10–50 ng of benzo[*a*]pyrene in the main stream smoke per cigarette, and the concentration is four times more in the side stream smoke (IARC 1986). In many PAH exposure

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reported to be a clear confounding factor to exposure assessments (Jongeneelen *et al.* 1989, Sherson *et al.* 1990, Grannela *et al.* 1993, VanRooij *et al.* 1993a). However, there are also reports where smoking did not have an effect on PAH exposure biomarkers (Burgaz *et al.* 1992, Omland *et al.* 1994). Another source of PAH exposure which also should be evaluated, according to many authors, when assessing environmental or occupational PAH exposure is the diet. Carcinogenic PAHs have been identified in many food items, especially in grilled or smoked meat and fish (IARC 1973, Fazio and Howard 1983). It has been estimated that dietary PAH exposure may contribute a considerable portion of the total PAH uptake (Santodonato *et al.* 1981, Fazio and Howard 1983, Lioy *et al.* 1988, Hattemer-Frei and Travis 1991, Buckley and Lioy 1992).

In this study we assessed the exposure of cokery workers to polycyclic aromatic hydrocarbons. To get a reliable measure of individual exposure, personal air sampling was conducted over a full shift. The use of skin wipe sampling was to get a semi-quantitative estimation of the possibility and contribution of exposure through the skin. We used 1-hydroxypyrene as a biomarker of internal dose as it has been shown to reflect external PAH exposure in many occupational environments. Control of the main confounding factors was conducted by a detailed questionnaire.

METHODS

Pyrene and β-glucuronidase/arylsulphatase (102.00 Units ml^{-1}) were obtained from Sigma Chemical Co., St Louis, MO; Benzo[*a*]pyrene was from Fluka Chemie AG, Buchs, SW; 1-hydroxypyrene was from Aldrich Chemical Co., Milwaukee, WI. All other chemicals were of analytical grade. C_{18} solid phase extraction cartridges were Mega Bond Elut® (6cc/1GRM) from Varian, Harbor City, CA; PTFE filters were obtained from Gelman Sciences, Ann Arbor, MA; XAD-2 adsorption tubes and Smear Tabs were from SKC Inc. Eighty Four, PA.

A total of 49 cokery workers from an Estonian oil shale plant at Kohtla-Järve and 10 controls from a nearby lisaku village took part in this study. All the persons participated voluntarily and were well informed about the purpose of the study beforehand. Sampling was conducted at the beginning of March 1994 and in September 1994. During the first 3-day sampling period a group of 22 coke oven workers and the controls were monitored. During the second sampling the size of the study group was 27 cokery workers. Eight persons took part in both of the samplings. Table 1 shows the distribution according to sex and smoking habits.

	Male		Female		
	Smokers	Non-smokers	Smokers	Non-smokers	Total
First sampling					
Cokery workers	11	2	1	8	22
Control persons	2	3	1	4	10
Second sampling					
Cokery workers	15	5	1	6	27

Table 1. Participants of the PAH exposure study.

Distribution of smoking was not similar in the exposed and control groups because of the strong sex-linkage of the smoking habit, and there were mostly male workers in the plant. The questionnaire (presented in native language) concerned personal characteristics including age, weight, height, smoking habits, alcohol consumption and work history. Furthermore, a medically trained person interviewed the participants of the study in connection with the blood sampling and checked the prefilled questionnaires. During the sampling, working activities, and thus possible exposure situations, were not followed by any industrial hygienist, but instead the gross evaluation of possible exposure level was based only on job titles mentioned in the questionnaire. There were 37 operators altogether, six locksmiths, three drivers, two loaders, and one welder in the total group of exposed workers studied. The information on the job title of two persons is missing. Eight of the control persons were white collar workers, one was a lumberman and one was a driver.

Full shift personal air samples were collected from the breathing zone of each worker, and the sampling was performed according to National Institute for Occupational Safety and Health (NIOSH) method 5506. Particulate matter was collected on a non-pre-equilibrated 37-mm-diameter polytetrafluoroethylene (PTFE) membrane filter which was followed by a glass sorbent tube containing 100/50 mg XAD-2 resin. The XAD-2 sorbent tube was wrapped with aluminium foil to prevent photodegradation of the PAHs collected. Air was drawn through the sampling media at the average rate of 1.5 l min^{-1} by charged constant flow personal air sampling pumps (Du Pont, Model P2500, and SKC, Model 224–42). The flow rates of the sampling pumps were calibrated beforehand and the calibrations were checked after sampling. Skin contamination was assessed from the inner surface of the wrist of every worker before and after the shift. The wrist skin was wiped by the sampler over an area twice the size of a Watman Smear tab (approx. 10 cm^2). Air samples and Smear tabs were wrapped in aluminium foil, transported in dry ice and kept at -70 °C until analysis. The concentration of urinary 1-OHP was measured from post shift spot samples.

Analysis of PTFE filters, XAD-2 adsorbent material and Smear tabs was performed according to a modification of NIOSH method 5506. The analytes were extracted in 5 ml of toluene: XAD-2 material in the desorption solvent was kept in a refrigerator overnight and after centrifugation a 4 ml aliquot was taken for further preparation. Filters and Smear tabs were extracted by sonication for 30 min. Air and skin samples were further handled in the same manner. After evaporation in nitrogen flow samples were redissolved in methanol, and analysed by HPLC using an Inertsil ODS-2 analytical column (3KI41013). HPLC equipment was a Millipore Waters™ 717 Autosampler, a 600-MS pump unit and a 470 Scanning fluorescence detector (Millipore Corp. Milford, MA). System control and data handling was done with a Millenium™ 2010 Chromatography Manager. Separation of analytes was by a linear gradient of acetonitrile/water (from 10%/90% in 25 min to 100%/0%). Fluorometric detection was at excitation wavelength 264 nm and at emission wavelength 381 nm for pyrene; and for B[*a*]P the wavelengths were 296 nm and 407 nm, respectively. Stability of detector response was controlled by repeating the injections of standard solution daily. Calibration curves were prepared by adding pyrene and B[*a*]P onto the filter and XAD-2, respectively. Urinary 1-OHP was analysed by high performance liquid chromatography after being submitted to enzymatic hydrolysis and solid phase clean-up (Jongeneelen *et al.*1987). The HPLC equipment was the same as for the air monitoring samples. Chromatographic conditions were as follows: a linear gradient of methanol/water (from 60%/40% in 25 min to 100%/0%). Fluorometric detection was at excitation wavelength 242 nm and at emission wavelength 388 nm. Stability of detector response was controlled by repeating the injections of standard solution daily. Calibration curves were made with blank urine samples spiked with known amounts of commercial 1-OHP. Creatinine concentration in each urine sample was determined from the urine collected during the workshift by a standard automated photometric method in the biomonitoring laboratory of the Finnish Institute of Occupational Health.

Variation coefficiency of pyrene analysis from the filters was 5±3(SD)% and recovery was 67±9(SD)%, *n*=3. For B[*a*]P analysis the variation coefficiency was 2±3(SD)% and recovery 75±5(SD)%. In the XAD-2 analysis the variation coefficiency for pyrene was 14±5(SD)% and for B[*a*]P 9±10(SD)%. The recoveries were 132±35(SD)% for pyrene and 110±21(SD)% for B[*a*]P. Variation coefficiences in Smear tab analyses were 4±3(SD)% for pyrene and 1±1(SD)% for B[*a*]P and recoveries were 76±45(SD)% and 97±20(SD)%, respectively. Variation coefficiency in urine analyses was 7±4(SD)% and recovery of 1-OHP was 137±10(SD)%.

	Sampling 1	Sampling 2	Both samplings
Kohtla-Järve			
Pyrene in filter samples (μg m^{-3})			
Range:	0.03–69.6	0.01–15.2	0.01–69.6
Mean:	15.7	1.5	8.1
Median:	4.8	0.1	0.2
B[*a*]P in filter samples (μg m^{-3})			
Range:	0.04–39.6	0.02–13.1	0.02–39.6
Mean:	10.4	1.6	5.7
Median:	3.7	0.2	0.4
Pyrene in skin wipe samples (ng cm^{-2})			
Range:	0.15–6.3	0.21–9.0	0.15–9.0
Mean:	2.0	1.3	1.6
Median:	1.1	0.7	0.8
B[*a*]P in skin wipe samples (ng cm^{-2})			
Range:	0–7.4	0–5.5	0–7.4
Mean:	2.0	0.7	1.3
Median:	1.1	0.2	0.3
Iisaku			
Pyrene in filter samples (μg m^{-3})			
Range:	0.001–0.004		
Mean:	0.002		
Median:	0.002		
B[*a*]P in filter samples (μg m^{-3})			
Range:	0–0.005		
Mean:	0.001		
Median:	0		
Pyrene in skin wipe samples (ng cm^{-2})			
Range:	0–0.46		
Mean:	0.12		
Median:	0		
B[*a*]P in skin wipe samples (ng cm^{-2})			
Range:	0		
Mean:	0		
Median:	0		

Table 2. Summary of personal hygienic PAH exposure measurements at the Kohtla-Järve cokery and in Iisaku village.

For statistical analyses of the study results Student's *t* test, Pearson correlation, and variance analyses were used. Results were examined also with log-normalization of 1-OHP values. The use of non-linear statistical tests did not change the results essentially.

Results

For the results of air concentration measurements and skin wipe sampling see Table 2 where the range of measured parameters is presented together with the mean and median values; the combination of the results from two separate measurements is also presented. In the cokery the pyrene concentration of the filter samples was 0.03–69.6 μg m^{-3} (mean 15.7 μg m^{-3}) in the first sampling, and 0.01–15.2 μg m^{-3} (mean 1.5 μg m^{-3}) during the second sampling. B[*a*]P concentration ranged from 0.04 to 39.6 μg m^{-3} (mean 10.4 μg m^{-3}) in the winter sampling and from 0.02 to 13.1 μg m^{-3} (mean 1.6 μg m^{-3}) in the autumn. In the control filter samples pyrene concentration was 0.001–0.004 μg m^{-3} (mean 0.002 μg m^{-3}) and B[*a*]P 0–0.005 μg m^{-3} (mean 0.001 μg m^{-3}). Benzo[*a*]pyrene was not found from the XAD-2 samples and only minimal amounts of pyrene were detected in the XAD-2 adsorbent material, less than 5% of the total pyrene load in most of the samples (in 71% of samples (*n*= 21) during the winter sampling and in 54% (*n*=24) of the second sampling). The statistically significant difference in airborne concentrations between sampling periods (*p*-value for pyrene=0.009 and for B[*a*]P=0.008) showed that based on occupational hygienic measurements by personal sampling the overall PAH exposure level was markedly higher during the first sampling period than the PAH levels measured in the air during the second period. Correlation between pyrene and B[*a*]P concentrations was very good (Figure 1). The measured pyrene contamination on wrist skin was 0.1–6.3 ngcm^{-2} (mean 2.0 ng cm^{-2}) and for B[*a*]P 0–7.4 ng cm^{-2} (mean 2.0 ng cm^{-2}) in the first sampling period. In the second sampling pyrene contamination ranged from 0.2 to 9.0 ng cm^{-2} (mean 1.3 ng cm^{-2}),

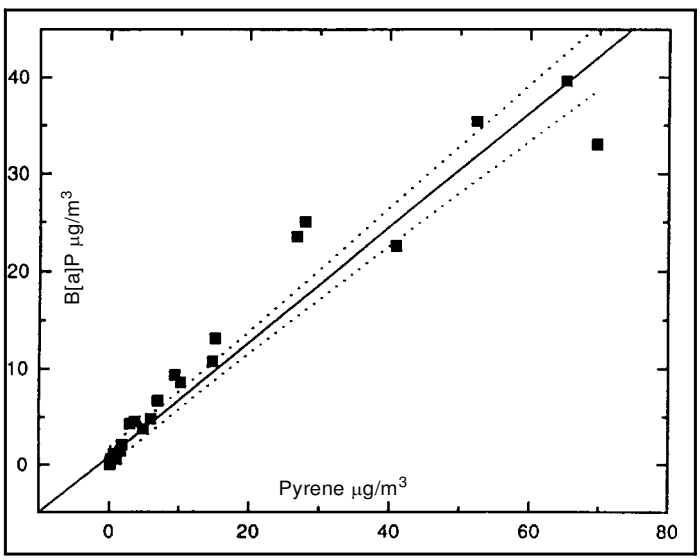


Figure 1. Correlation between pyrene and benzo[*a*]pyrene measured in personal air samples of cokery workers. A linear regression with 95% confidence limits is shown. *n*=45; *r*=0.97; *p*<0.00001.

	1-OHP (μmol mol^{-1} creatinine)	1-OHP (nmol l^{-1})
Sampling 1		
Exposed		
Range:	0.2–69.5	1.2–409.8
Mean:	8.7	72.9
Median:	3.4	32.7
Sampling 2		
Exposed		
Range:	0.28–21.6	2.2–354.8
Mean:	3.7	61.6
Median:	1.5	19.9
Controls		
Range:	0.13–1.68	1.65–19.0
Mean:	0.5	6.3
Median:	0.3	4.3

Table 3. Urinary 1-hydroxypyrene (1-OHP) concentrations in cokery workers and controls.

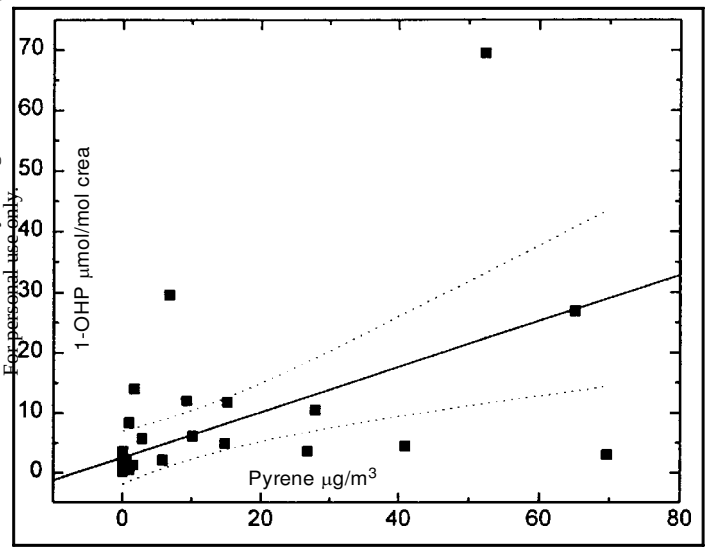


Figure 2. Correlation between the pyrene concentration in personal air samples and urinary 1-hydroxypyrene. A linear regression with 95% confidence limits is shown. n=41; r=0.56; p=0.0002.

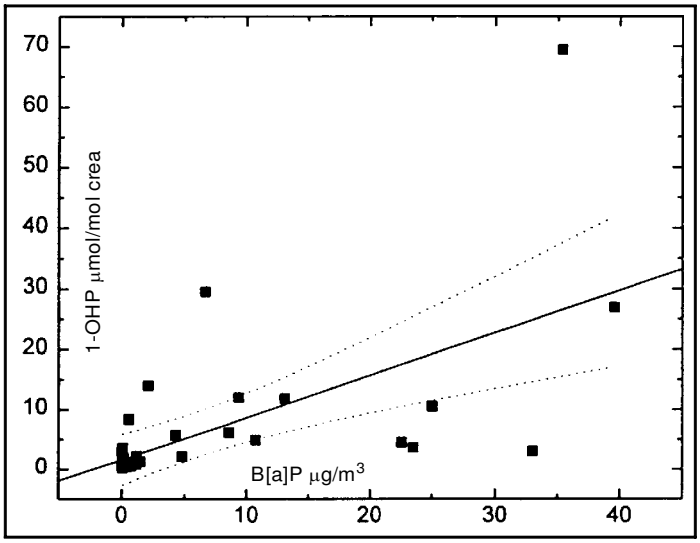
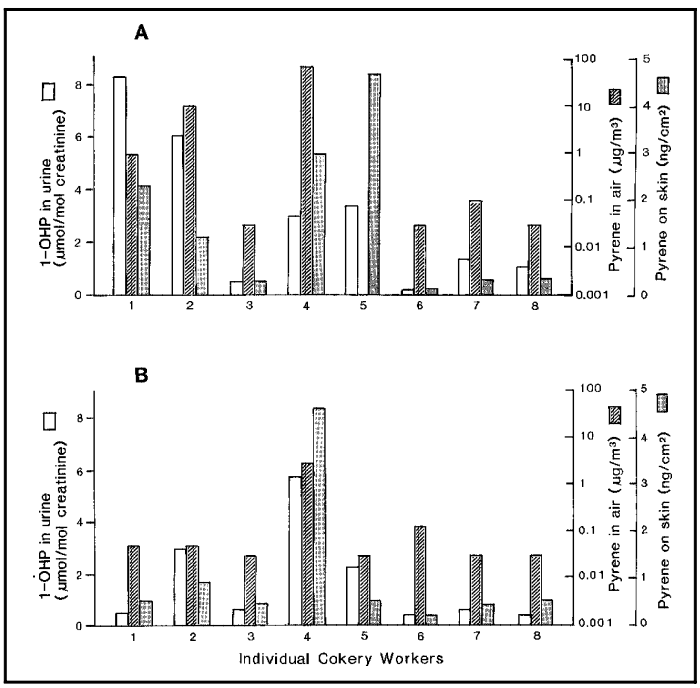


Figure 3. Correlation between the B[*a*]P concentration in cokery filter samples and urinary 1-hydroxypyrene. A linear regression with 95% confidence limits is shown. n=41; r=0.63; p=0.00001.



Figures 4A. and 4B. 1-Hydroxypyrene concentration in urine (μmol/mol creatinine), pyrene concentration in breathing zone air (μg m^{3}), the numerical value multiplied by 10, and pyrene contamination on skin (ng cm^{-2}) in eight cokery workers in two separate samplings. Figure 4A is from the winter sampling and figure 4B from the autumn sampling. Person No 5 in Figure 4A missed the air measurement result.

and B[*a*]P contamination from 0 to 5.5 ng cm^{-2} (mean 0.7 ng cm^{-2}), respectively. A clear skin contamination was demonstrated and in most cases (60%, n=48) the postshift value was higher although handwashing could not be controlled in all cases before sampling.

1-Hydroxypyrene was measured from post shift urine samples of 49 exposed workers, and the 10 controls collected a sample during the same day as air monitoring was conducted. Most of the studied subjects were smokers (75%). In our study, the controls excreted levels of 1-hydroxypyrene showing a mean of 0.5 μmol mol^{-1} creatinine (6.3 nmol l^{-1}). The exposed workers showed clearly elevated levels of 1-hydroxypyrene in their urine, the mean value being 6.0 μmol mol^{-1} creatinine (66.8 nmol)$^{-1}$, range 0.2–69.5 μmol mmol^{-1} creatinine (1.2–409.8 nmol l^{-1}). The range, mean and median values of 1-OHP concentrations are given in Table 3. Correlation between airborne pyrene and 1-hydroxypyrene (expressed with creatinine adjustment) was strong, showing an r-value of 0.56 (Figure 2). The corresponding r-value was 0.52 when the 1-OHP concentration was not corrected for creatinine. Correlation between B[*a*]P and 1-hydroxypyrene was even stronger, showing an r-value of 0.63 (Figure 3). Figures 4A and 4B show the comparison of airborne pyrene concentration

measurements, skin wipes and urinary 1-OHP concentration in the eight cokery workers who participated in both samplings. Higher 1-OHP concentrations seem to co-exist with high air concentations of pyrene, and also the effect of heavy skin contamination is generally demonstrated as a high 1-OHP concentration. Despite this obvious tendency, the separate contribution of these different components could not be statistically verified in this small number of cases.

In our study all the assessed contributors of pyrene dose: occupational exposure through inhalation, absorption through the skin and smoking had a statisitically significant effect on the measured 1-OHP concentrations, the corresponding p-values were 0.007; 0.012; 0.022 (creatinine adjusted 1-OHP with log-normalization) and 0.014; 0.005; 0.012, respectively with log-normalized 1-OHP expressed in nmol l^{-1}. The effect of smoking was seen when this parameter was expressed as ±. The effect of packyear could not be statistically seen in this study.

Discussion

In this study pyrene and benzo[*a*]pyrene were selected to be measured as markers of overall PAH exposure because of the known carcinogenicity of benzo[*a*]pyrene, and pyrene as being an important component of all PAH mixtures, and because the main urinary metabolite of pyrene was determined as a marker of internal dose. In this study we found that the cokery workers were exposed to high concentrations of pyrene and benzo[*a*]pyrene. The results of biological monitoring are in agreement with occupational hygiene measurements from the workplace atmosphere. In our study the correlation between airborne pyrene and benzo[*a*]pyrene (r=0.97, p<0.00001) was extremely good, and is in agreement with results in other studies where fairly constant ratios of pyrene to other PAHs in specific work environments, such as coke plants, have been reported (Jongeneelen *et al.* 1990, Buchet *et al.* 1992).

The exposure level at the Kohtla-Järve coke plant is clearly higher (the overall mean value for B[*a*]P concentration 5.7 μg m^{-3}) than in modern cokeries. In a Finnish cokery study Yrjänheikki *et al.* reported (1994) B[*a*]P concentrations in personal samples at the level of 2.0 μg m^{-3}. In another recent study topside cokery workers were exposed to pyrene concentrations of 9.44 (GM) μgm^{-3} and to B[*a*]P concentrations of 5.85 (GM) μg m^{-3} (Buchet *et al.* 1992). Mean concentrations of the pyrene and benzo[*a*]pyrene were markedly higher (for pyrene 15.7 μg m^{-3} versus 1.5 μg m^{-3} and for B[*a*]P 10.4 μg m^{-3} versus 1.6 μg m^{-3}) during the winter sampling compared with the concentration levels in the autumn. This observation is in agreement with the general phenomenon that air concentrations of PAHs are generally higher in the environment during the cold season. Higher 1-OHP concentrations have also been shown in winter time with good correlations between 1-OHP and airborne PAH concentrations (Zhao *et al.* 1990, 1992). The same tendency was also demonstrated in our study (although the difference was not statistically significant in the t-test) which supports our interpretation; and at least partly excludes the possibility of gross bias in sampling or that it is all chance. The less clear effect on 1-OHP concentrations could partly be explained by the fact that numerous other sources of pyrene, as well as air, were having an effect on this biomarker.

1-OHP is considered to be a suitable biomarker of occupational/environmental PAH exposure for several reasons: firstly many studies have shown a reasonable correlation to markers of external exposure (Zhao *et al.* 1990, Grimmer *et al.* 1993, Levin *et al.* 1995, Quinlan *et al.* 1995); and secondly, the relative simplicity and rapidity of the assay and the non-invasiveness of specimen collection make the assay amenable to routine sampling. In a recent study by Clonfero *et al.* (1995) the power of 1-OHP over urinary mutagenicity as a sensitive marker of occupational exposure to PAHs was demonstrated. Also, a recent international workshop concluded that urinary 1-hydroxypyrene is a useful tool for estimation of occupational PAH exposure. However, since there is no direct correlation between urinary 1-hydroxypyrene and health effects, it cannot yet be used as an indicator of risk (Levin 1995).

In our study most of the subjects were smokers (75%). As showed by Van Rooij *et al.* (1994a) smoking and dietary intake of PAH have an effect on the baseline excretion of 1-OHP in urine. In our study the mean value of 1-OHP excretion in the controls (0.5 μmol mol^{-1} creatinine) falls in the range of control values for 1-OHP excretion, reviewed recently by Levin (1995). The exposed workers showed clearly elevated levels of 1-OHP in their urine. The 1-OHP concentration was about 12 times higher (mean 6.0 μmol mol^{-1} creatinine, range 0.2–69.5 μmol mol^{-1} creatinine) than for the local control population. This difference is in the same range as reported in other previous studies (Jongeneelen *et al.* 1990, Kang *et al.* 1995). In a recent study of Øvrebø *et al.* (1995) the mean urinary 1-hydroxypyrene concentration in samples from coke oven workers was reported to vary from 1.11 to 5.33 μmol mol^{-1} creatinine and the average value for the controls was 0.14 μmol mol^{-1} creatinine. In this study the external exposure was assessed according to job category only.

The use of creatinine correction in order to reduce the variations due to dilution is commonly used in connection with expression of urinary metabolite concentrations. There has been criticism toward the use of creatinine adjustment. It has been shown that considerable intra- and interindividual variation exists in the excretion of creatinine, the excretion rate being non-constant and unpredictable (Alessio *et al.* 1985, Boeniger *et al.* 1993). In this study the correlation between airborne pyrene concentration and 1-OHP did not differ considerably while the biomarker was expressed as nmoll^{-1} (r=0.52, p=0.0005) or adjusted for creatinine, μnmol/mol creatinine (r=0.56, p=0.0002). The effect of adjustment should be tested and compared with non-adjusted values in individual situtations. In the case of marked discrepancy, after critical consideration of the underlying reasons, the best possible way of expressing the results should be chosen.

Several studies have shown that 1-OHP excretion is influenced by smoking only at very low occupational exposure levels (Jongeneelen *et al.* 1988b, Granella and Clonfero 1993, VanRooij *et al.* 1994b,); while in high exposure situations, as in coke oven work, only a minimal effect of smoking on 1-OHP excretion has been observed (Buchet *et al.* 1992). Also

Jongeneelen *et al.* (1988a) concluded that high occupational exposure masks the increase of 1-OHP excretion due to smoking related pyrene intake. In this study group, smoking was shown to have a statistically significant impact on 1-OHP level.

In our study diet was not controlled as a source of total PAH exposure. Presumably, in this cohort the effect of dietary PAH load is smaller than that of smoking. No doubt the recent statement by Buckley *et al.* (1995) holds true: in low or moderate exposure situations, environmental or occupational, epidemiological or exposure/biomarker studies that fail to consider dietary PAH exposure are likely to result in serious misclassification of exposure.

We used skin wipe sampling in order to assess semi-quantitatively the possible impact of skin as a route for PAH exposure. Results from the skin wipe sample analyses clearly supported the visual observations of heavy contamination on skin and clothing. This observation is an important one when considering protective hygienic measures, also because in other studies the skin has been shown to be one of the main routes of exposure to PAHs in a coke plant and in coal tar creosote impregnation (VanRooij *et al.* 1993a, 1993b). Data on the skin contamination of workers exposed to PAHs are still very limited. Coveralls may reduce skin contamination, but they also may increase the dermal absorption rate of contaminants due to the elevated temperature of the skin, humidity, and physical stress (VanRooij *et al.* 1993c). In a recent study it was demonstrated that simple hygienic skin protective measures result in a significant reduction (37%) of the internal PAH exposure (VanRooij *et al.* 1994b).

The power of biomarkers in risk-based assessment of exposure is based on their biological relevance as providing indisputable evidence of exposure, being an integral over time and route of exposure (Buckley *et al.* 1995). However, biomarker measurement provides no information about the source of exposure or route of absorption. Therefore in this study external PAH exposure also was assessed by traditional occupational hygiene measurements combined with a biomonitoring technique in order to get a comprehensive picture of the total exposure. When interpreting the results of air sampling and assessing the exposure to compounds via the lungs it should be kept in mind that the interindividual variation in respiration and the use of protective equipment have an effect on the actual amount of internal dose deriving from the atmosphere (Ny *et al.* 1993). Also the actual bioavailability depends on the nature of the inspired particles.

Assessment of PAH exposure through the skin is clearly a new challenge for occupational hygienists, and standardized methods are still lacking. However, recent observations of PAH absorption through the skin, possible exposure from contaminated clothing, and the significant exposure-reducing effect of protective clothing and other hygienic measures provide support for practical measures towards better working conditions (VanRooij *et al.* 1992, 1993a, 1994, Boogaard and van Sittert 1995, Quinlan *et al.* 1995).

In conclusion, in this study we found that the studied cokery workers were exposed to high concentrations of pyrene and benzo[*a*]pyrene. Results of biological monitoring are in agreement with occupational hygiene measurements from the workplace atmosphere. Skin contamination and hence the importance of possible PAH exposure through the skin is obvious. Encouraged by recent study results, occupational hygienic measures should also be directed to reduce skin contamination. There was a clear difference of 1-hydroxypyrene concentrations in controls compared with exposed workers and a strong correlation between occupational exposure to pyrene and urinary 1-hydroxypyrene level, which justifies the use of 1-hydroxypyrene as a marker of occupational PAH exposure. In this study, the contribution of smoking-related pyrene load was demonstrated in statistical analyses as causing a clear difference in the baseline level of 1-hydroxypyrene excretion in smokers versus non-smokers.

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References

Alessio, L., Berlin, A., Dell'Orto, A., Toffoletto and F., Ghezzi, I. (1985) Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. *International Archives of Occupational and Environmental Health*, **55**, 99–106.

Bjørseth, A., Bjørseth, O. and Fjeldstad, P.E. (1978) Polycyclic aromatic hydrocarbons in the work atmosphere. II. Determination in a coke plant. *Scandinavian Journal of Work, Environment & Health*, **4**, 224–236.

Boeniger, M. F., Lowry L.K. and Rosenberg, J. (1993) Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *American Industrial Hygiene Association Journal*, **54**, 615–627.

Boogaard, P.J. and van Sittert, N.J. (1995) Urinary 1-hydroxypyrene as biomarker of exposure to polycyclic aromatic hydrocarbons in workers in petrochemical industries: baseline values and dermal uptake. *Science of the Total Environment*, **163**, 203–209.

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.

Buckley, T.J. and Lioy P.J. (1992) An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. *British Journal of Industrial Medicine*, **49**, 113–124.

Buckley, T. J., Waldman, J. M., Dhara, R., Greenberg, A., Ouyang, Z. and Lioy, P. J. (1995) An assessment of a urinary biomarker for total human environmnetal exposure to benzo[a]pyrene. *International Archives of Occupational and Environmental Health*, **67**, 257–266.

Burgaz, S., Borm, B.J.A, and Jongeneelen, F.J. (1992) Evaluation of urinary excretion of 1-hydroxypyrene and thioethers in workers exposed to bitumen

fumes. *International Archives of Occupational and Environmental Health*, **63**, 397–401.

CLONFERO, E,. GRANELLA, M., MARCHIORO, M., BARRA, E.L., NARDINI, B., FERRI, G. AND FOÀ, V. (1995) Urinary excretion of mutagens in coke oven workers. *Carcingenesis*, **16**(3): 547–554.

DUFRESNE, A., LESAGE, J. AND PERRAULT, G. (1987) Evaluation of occupational exposure to mixed dusts and polycyclic aromatic hydrocarbons in silicon carbide plants. *American Industrial Hygiene Association Journal*, **48**, 160–166.

ELOVAARA, E., HEIKKILÄ, P., PYY, L., MUTANAEN, P. AND RIIHIMÄKI, V. (1995) Significance of dermal and respiratory uptake in creosote workers: exposure to polycyclic aromatic hydrocarbons and urinary excretion of 1-hydroxypyrene. *Occupational and Environmental Medicine*, **52**, 196–203.

FAZIO, T. AND HOWARD, J.W. (1983) Polycyclic aromatic hydrocarbons in foods. In *Handbook of Polycyclic Aromatic Hydrocarbons*, Vol. 1, A. Bjorseth, ed. (Marcel Dekker, New York), pp. 461–505. .

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., 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.

HATTEMER-FREI, H.A. AND TRAVIS, C. C. (1991) Benzo(*a*)pyrene; environmental partitioning and human exposure. *Toxicology and Industrial Health*, **7**, 141–157.

IARC: International Agency for Research on Cancer (1973) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. *Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Components*, Vol. 3 (IARC, Lyon), pp. 91–136.

IARC: International Agency for Research on Cancer (1984) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Polynuclear Aromatic Compounds. Industrial Exposures in Aluminum Production, Coal Gasification, Coke Production, and Iron Steel Founding*, Vol. 34, part 3 (IARC, Lyon), pp. 101–131.

IARC: International Agency for Research on Cancer (1986) IARC Monograph on the Evaluaton of Carcinogenic Risks to Humans. *Tobacco Smoking*, Vol. 38 (IARC, Lyon), pp. 83–126.

IARC: International Agency for Research on Cancer (1987) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Overall Evaluations of Carcinogenicity. An Updating of IARC Monographs Vols 1–42*, Supplement 7 (IARC, Lyon) pp. 174–177.

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., ANZION, R.B.M., SCHEEPERS, P.T.J., BOS, R.P., HENDERSON, P.TH., NIJENHUIS, E. H., VEENSTRA, S.J., BROUNS, R.M.E. AND WINKES, A. (1988b) 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., SCHEEPERS, P.T. J., GROENENDIJK, A., VAN AERTS, L.A.G.J.M., ANZION, R.B.M., BOS, R.P. AND VEENSTRA, S.J. (1988a) Airborne concentrations, skin contamination, urinary metabolite excretion of polycyclic aromatic hydrocarbons among paving workers exposed to coal tar derived road tars. *American Industrial Hygiene Association Journal*, **49**, 600–607.

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.

JONGENEELEN, F.J., ANZION, R.B.M. AND HENDERSON, P.TH. (1987) Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *Journal of Chromatography*, **413**, 227–232.

JÜRGENFELD, G., LOOG, T. AND VAHER, E. (1994) Oil shale. In *Estonian Mineral Resources*, (Ministry of the Environment of Estonia, Environment information Centre, Tallinn), pp. 10–18.

KANG, D., ROTHMAN, N., CHO, S.-H., LIM, S.H., KWON, H.-J., KIM, S.M., SCHWARTZ, B. AND STRICKLAND, P.T. (1995) Association of exposure to polycyclic aromatic hydrocarbons (estimated from job category) with concentration of 1-hydroxypyrene glucuronide in urine from workers at a steel plant. *Occupational and Environmental Medicine*, **52**, 593–599.

LESAGE, J., PERRAULT, G. AND DURAND, P. (1987) Evaluation of worker exposure to polycyclic aromatic hydrocarbons. *American Industrial Hygiene Association Journal*, **48**, 753–759.

LEVIN, J.O. (1995) First international workshop on hydroxypyrene as a biomarker for PAH exposure in man-summary and conclusions. *Science of the Total Environment*, **163**, 165–168.

LEVIN, J.O., RHÉN AND M., 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.

LIOY, P.L., WALDMAN, J.M., GREENBERG, A., HARKOV, R. AND PIETARINEN, C. (1988) The total human environmental exposure study (THEES) to benzo(*a*)pyrene: comparison of the inhalation and food pathways. *Archives of Environmental Health*, **43**(4), 304–312.

NY, E.T., HEEDERIK, D., KROMHOUT, H. AND JONGENEELEN, F. (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.

OMLAND, Ø., SHERSON, D., HANSEN, Å.M., SIGSGAARD, T., AUTRUP, H. AND OVERGAARD, E. (1994) Exposure of iron foundry workers to polycyclic aromatic hydrocarbons: benzo(*a*)pyrene–albumin adducts and 1-hydroxypyrene as biomarkers for exposure. *Occupational and Environmental Medicine*, **51**, 513–518.

ØVREBØ, S., HAUGEN, A., FARMER, P.B. AND ANDERSON, D. (1995) Evaluation of biomarkers in plasma, blood, and urine samples from coke oven workers: significance of exposure to polycyclic aromatic hydrocarbons. *Occupational and Environmental Medicine*, **52**, 750–756.

QUINLAN, R., KOWALCZYK, G., GARDINER, K. AND CALVET, I. (1995) Exposure to polycyclic aromatic hydrocarbons in coal liquefaction workers: impact of a workwear policy on excretion of urinary 1-hydroxypyrene. *Occupational and Environmental Medicine*, **52**, 600–605.

REUTERWALL, C., ARINGER, L., ELINDER, C.-G., RANNUG, A., LEVIN, J.-O., JURINGE, L. AND ÖNFELT, A. (1991) Assessment of genotoxic exposure in Swedish coke-oven work by different methods of biological monitoring. *Scandinavian Journal of Work, Environment & Health*, **17**, 123–132.

ROOKS, I. (1994) A historical review of RAS "Kiviter". *Oil Shale*, **11**, 283–285.

SANTODONATO, J., HOWARD, P. AND BASU, D. (1981) Health and ecological assessment of polynuclear aromatic hydrocarbons. *Journal of Environmental Pathology, Toxicology and Oncology*, **5**, 1–376.

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.

VANROOIJ, J.G.M., BODELIER-BADE, M.M., HOPMANS, P.M.J. AND JONGENEELEN, F.J. (1994b) Reduction of urinary 1-hydroxypyrene excretion in coke oven workers exposed to polycyclic aromatic hydrocarbons due to improved hygienic skin protective measures. *Annals of Occupational Hygiene*, **38**, 247–256.

VANROOIJ, 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.

VANROOIJ, J.G.M., VANLIESHOUT, 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, & Health*, **19**, 200–207.

VANROOIJ, J.G.M., VEEGER, M.M.S., BODELIER-BADE, M.M., SCHEEPERS, P.T.J. AND JONGENEELEN, F.J. (1994a) 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

VanRooij, J.G.M., Bodelier-Bade, M.M., De Looff, A.J.A., Dijkmans, A.P.G. and Jongeneelen, F.J. (1992) Dermal exposure to polycyclic aromatic hydrocarbons among primary aluminum workers. *Medicina del Lavoro*, **83**, 519–529.

Yrjänheikki, E., Pyy, L., Hakala, E., Lapinlampi, T., Lisko, A. and Vähäkangas, K. (1994) PAH-altistuminen uudessa koksaamossa. *Työympäristötutkimuksen aikakaus-kirja. Työ ja ihminen*, **8**, 209–217 (in Finnish).

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.

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