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Determination of Exposure to Polycyclic Aromatic Hydrocarbons in Some Work Groups in Turkey by Measurement of 1-Hydroxypyrene Levels in Urine

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Polycyclic aromatic hydrocarbons (PAHs) are a well-known and widely used important class of environmental contaminants that exert various effects on humans (IPCS 1998). They are naturally occurring environmental chemicals that result from the incomplete combustion of fossil fuels such as coal and the burning of various substances as a primary source. Exposure to these chemicals usually occurs as exposure to mixtures and not to individual chemicals. It is reported that tens of thousands of tons of PAHs are released into the atmosphere each year in USA and as a result it leads to contamination of air, water and soil (Zedeck 1980). PAHs are found in motor vehicle exhaust, residential and industrial furnaces, tobacco smoke and volcanoes and by-products of coal tar, coke oven emissions, bitumen fumes, industrial smoke, tobacco and cigarette smoke and charcoal-broiled foods so human exposure to PAHs is therefore unavoidable. It is a well-known fact that some of the occupations include working in coke production, working as chimney sweeps, asphalt applicators are exposed to PAHs from the burning or coking of petroleum products more often than the other people (IARC 1984; IARC 1985; IARC 1987). PAHs have long been known to cause cancer in animals and are commonly believed to significantly contribute to human cancers. They have been widely classified as known human carcinogens. Several PAHs can produce cancer in experimental animals, and epidemiological studies of exposed workers, especially in coke-ovens and aluminium smelters have shown clear excesses of lung cancer and highly suggestive excesses of bladder cancer (Boffetta et al. 1997; IARC 1984; IARC 1985; IARC 1987; Negri and La Vecchia 2001). PAHs are also known to cause skin, breast, lymphoma and testicular cancer and other diseases in various organ systems (Burchiel and Luster 2001). In order to identify the persons in particular at risk of developing those cancers, it is essential to establish the type and amount of carcinogen absorbed, by evaluating concentrations of the carcinogen or its metabolites in body fluids, which leads to identification of exposure markers. As biomarker of the absorbed dose of aromatic hydrocarbons, urinary 1-hydroxypyrene (1-OHP) is commonly used.

As another source of PAHs, bitumens are principally used in road construction especially in asphalting, in roofing felt manufacture and pipe coating. They are viscous solids or liquids derived from refining process of petroleum and classified

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as possible human carcinogens (IARC Group 2B) (IARC 1987). In Turkey, it is estimated that the current annual use of bitumens is approximately more than 0.35 million tons. Fumes of bitumens contain PAHs so the asphalt workers have a risk of exposure. In Turkey, asphalting season starts near the beginning of summer and ends at or near the end of it. Road operations and asphalting becomes intense in this season.

This study was designed to assess the exposure levels of workers employed in different work branches having high risk of PAH exposure in Turkey. Urinary 1-OHP excretions were used as a biomarker of occupational exposure. It was also aimed to evaluate a possible effect of smoking habit on urinary 1-OHP levels measured.

MATERIALS AND METHODS

In this study, three different groups of workers and a control group were selected. The two exposed groups were working as asphalt workers exposed to bitumen fumes containing PAHs and the other group was coke oven workers. The aim of taking two groups of asphalt workers was to find out whether the sampling time has an augmenting contribution on the effects of PAH exposure. The first group of 10 asphalt workers was sampled at the beginning of asphalt season in June and the second group of 16 workers was sampled at nearly the end of the asphalt season in September. All the subjects provided approximately 35-40 ml of urine samples that were collected at the end of their workshifts on the last day of a routine working week. Urine, protected from light, was stored at –20 °C until analyses. In addition, each employee responded to a brief questionnaire that gives information about occupational histories, drug usage, smoking and drinking habits. A group of 15 age-matched healthy workers with no history of occupational PAH exposure was used as control.

Urinary 1-OHP was analyzed according to the method developed by Jongeneelen et al. (1985). The determination was based on the enzymatic hydrolysis of the conjugated metabolites and their solid-phase extraction. 10 ml of urine sample was adjusted at pH 5.0 with 1M HCl and 0.1 M acetate buffer pH 5.0 was added to a volume of 30 ml. The mixture was hydrolyzed enzymatically with 12.5 µl of β-glucuronidase/arylsulphatase at 37 °C for 16 h. Afterwards, the hydrolyzed urine sample was applied to C18 reverse-phase cartridge (Extract-Clean/RC 500 mg C18, Alltech Associate Inc., IL, USA), which had been washed with 5 ml of methanol followed by 10 ml of distilled water. The hydrolyzed sample was passed through the cartridge and had been washed with 10 ml of water. The retained solutes were eluted with 9 ml of methanol. The solvent was evaporated at 50 °C under gentle flow of nitrogen. The residues were resolved in 2 ml of methanol in an ultrasonic bath for 4 min. Afterwards, it was centrifuged at 600xg for 5 min then the sample was taken into a capped vial before application into the HPLC. 5 µl of sample was injected into HPLC system (Hewlett Packard Series 1100, USA) with a Hypersil ODS (5 µm) column.

1-OHP was quantitatively determined by use of a fluorescence detector with excitation and emission wavelengths of 242 and 388 nm, respectively. The column temperature was 40^0C and the flow rate was 0.8 ml/min. The solvent gradient was as follows: 5 min of methanol/water (57:43), a linear gradient of 22 min to methanol/water (69:31) and 24 min of (57:43). 1-OHP gave a peak at 17.0 min. Peak height was used for quantification. The detection limit for 1-OHP was 0.07 nmol/l. Urinary 1-OHP concentrations were corrected by creatinine concentrations and were expressed as μmol/mol creatinine.

Statistical analyses were performed using SPSS version 9.0. To test for statistical significant differences among three exposed groups and control subjects, the analysis of variance (ANOVA) for the means of urinary 1-OHP was used. Bonferroni's multiple range test was used for multiple comparison of means after ANOVA with statistically significant difference was found.

RESULTS AND DISCUSSION

The general characteristics of the control and the exposed groups are summarized in Table 1. 1-OHP was used as a biomarker as it has been shown to reflect external PAH exposure in many occupational environments. As it was shown in the table, the mean urine 1-OHP level of the control group was 0.40±0.05 μmol/ mol creatinine and that of the PAH-exposed groups are 0.51±0.16 (first asphalt group), 0.939±0.13 (second asphalt group), 0.98±0.18 μmol/ mol creatinine (oven workers) respectively. A statistically significant enhancement was found in the 1-OHP levels of oven workers and those of the control groups ($P<0.01$). Although a significant difference was found between the 1-OHP levels of the second asphalt group and those of the control ($P<0.01$), there was a slight but not significant enhancement in the first asphalt group as compared to control. The urinary levels of 1-OHP of the control and the other PAH-exposed groups were also shown in Figure 1. There was no significant difference between the 1-OHP levels of the two asphalt groups although there was an increase in the second asphalt group compared with the first one. This result points out that the sampling time may play a role in the assessment of PAH-exposure.

In this study, it was also aimed to evaluate whether a possible effect of smoking habit on urinary 1-OHP levels measured could be observed or not. The 1-OHP results were subgrouped into those of smokers and non-smokers in control and PAH-exposed individuals separately. Although there was no statistically significance between asphalt groups and control groups on the basis of smoking, urinary 1-OHP levels of the smoker oven workers showed a statistically significant increase than those of the both smoker ($P<0.05$) and nonsmoker ($P<0.01$) control group. Effects of smoking between the 1-OHP levels of control and exposed workers were illustrated in Table 2.

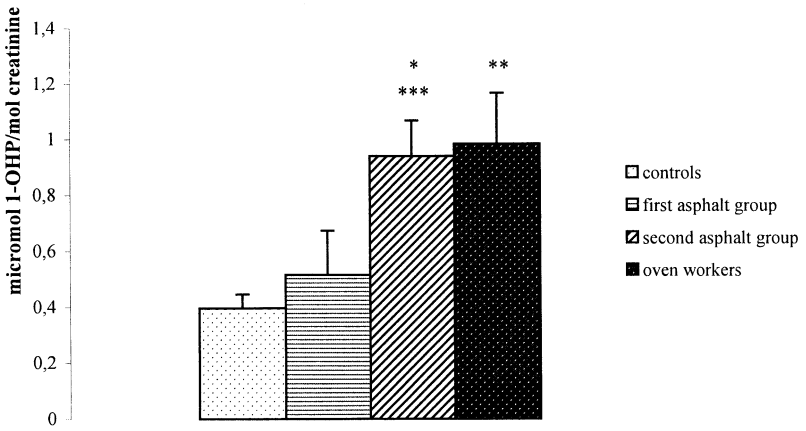

Figure 1. Comparison of urinary 1-OHP levels (±S.E.) of control and exposed groups

* P<0.01 versus control, ** P<0.01 versus control, *** P<0.05 versus first asphalt group

As PAHs become a more threatening subject in human health day by day because of their worldwide excessive exposure, the determination of PAHs by measurement of their metabolites acquires importance also. Air monitoring of PAHs only quantifies the respiratory intake. PAHs may be absorbed not only in the lungs, but also in the gastrointestinal track and through the skin. The monitoring of PAHs or their metabolites in body fluids reflects the total uptake (internal dose). Measurement of PAH metabolites in human urine constitutes a well-accepted means for assessing individual internal doses of PAHs. 1-OHP, which accounts for about 60-80% of pyrene derivatives excreted in urine of all mammalian species studied so far, including humans, appears to be a normal urinary metabolite in people with recent exposure to different originated PAHs (Viau et al. 1999). The relative simplicity and rapidity of the assay and the non-invasiveness of specimen collection make the assay amenable to routine sampling makes 1-OHP to be considered as a suitable biomarker also.

Table 1. Age, duration of exposure, smoking habit, urinary 1-OHP levels of control and exposed groups

	Controls (n=15)	First asphalt group (n=10)	Second asphalt group (n=16)	Oven workers (n=13)
Age (mean, range)	40.66 (33-47)	38.50 (32-45)	37.00 (29-43)	44.50 (40-48)
Duration of exposure (years,mean, range)	-	12.90 (4-22)	9.9 (2-17)	20.08 (17-25)
Active smokers (percentage of population)	6 (%40)	6 (%60)	9 (%56)	9 (%69)

The main object of this study was to evaluate the different exposure levels of various groups of workers exposed to PAHs by measurement of urinary 1-hydroxypyrene (1-OHP) levels as a biomarker of occupational exposure. All the 1-OHP results were corrected for creatinine in order to reduce the variations due to the dilution. The results showed that a statistically significant enhancement was found in the 1-OHP levels of oven workers and those of the control groups ($P<0.01$). The 1-OHP concentrations of the oven workers was approximately 2.5 fold higher than those of the controls. Various researchers found several times higher 1-OHP concentrations in coke oven workers compared to those of the controls (Kuljukka et al. 1997; Lu et al. 2002).

Table 2. Effects of smoking between the 1-OHP levels of control and exposed workers

	Urinary 1-OHP level (mean±S.E., μmol/ mol creatinine)			
	Controls	First asphalt group	Second asphalt group	Oven workers
Non smokers	n=9	n=4	n=7	n=4
1-OHP levels	0.36 ± 0.06	0.38 ± 0.10	0.98 ± 0.21	0.45 ± 0.24
Smokers	n=6	n=6	n=9	n=9
1-OHP levels	0.44 ± 0.10	0.65 ± 0.30	0.91 ± 0.17	1.22 ± 0.20[a,b]

[a] $P< 0.01$ versus nonsmoker controls, [b] $P< 0.05$ versus smoker controls

In various studies researching the evaluation of PAH exposure in asphalt workers by measuring 1-OHP levels in urine have shown that a significant difference in 1-OHP levels of the exposed workers compared to controls which are supporting the results we found in this study (Burgaz et al. 1992; Jarvholm et al. 1999; Jongeneelen et al. 1988; Szaniszlo and Ungvary 2001). In our study the difference between the control and the second asphalt group was approximately 2.5 fold. Although a significant difference was found between the 1-OHP levels of the second asphalt group and those of the control ($P<0.01$), there was a slight but not significant enhancement in the first asphalt group compared to control (Fig. 1). It was found that there was an increase (almost 2 fold) in the second asphalt group compared with the first one ($P<0.05$). This may be due to the extensive PAH exposure of the workers during the summer season. This result points out that the sampling time may play a role in the assessment of PAH-exposure. For the further studies, sampling time may be an important criterion that has to be considered during designing the study.

In this study, it was also aimed to evaluate a possible effect of smoking habit as a confounding factor on urinary 1-OHP levels measured. Several studies have shown that 1-OHP excretion is influenced by smoking only at very low occupational exposure levels (Grannela and Clonfero 1993; Van Rooij et al. 1994) 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). We found a statistically significant difference in only smoker oven workers compared to both smoker and non-smoker controls (Table 2). There was a graded but not significant enhancement especially in the levels of the smokers, from the control to the oven

workers as it was shown in Table 2. Szaniszlo and Ungvary (2001) reported that smoking is a decisive factor in PAH exposure but in contrast, our study revealed that smoking can be a considerable factor but not a strong determinant in evaluation of PAH exposure in workers. This may be due to the inadequate number of the samples. Also, working place conditions must be considered as a confounding factor in exposure.

The results we observed from our study demonstrated that urinary 1-OHP is a suitable biomarker for assessing PAH exposure and its measurements can be considered reliable for biological monitoring. Smoking habit and job category of the workers and the sampling time are considerable confounding factors that may affect their level of exposure and the excretion of urinary 1-OHP.

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