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# Biological Monitoring of Environmental Exposure to Polycyclic Aromatic Hydrocarbons: 1-Hydroxypyrene in Urine of Turkish Coke Oven Workers

M. Yilmazer,[1] A. O. Ada,[2] S. Suzen,[2] C. Demiroglu,[1] A. E. Demirbag,[3] S. Efe,[4] Y. Alemdar,[4] M. Iscan,[2] S. Burgaz[1]

[1] Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, Turkey
[2] Department of Toxicology, Faculty of Pharmacy, University of Ankara, Ankara, Turkey
[3] Gastrointestinal Surgery Department, Yuksek Ihtisas Hospital, Ankara, Turkey
[4] Eregli Iron and Steel Works Co., Karadeniz Eregli, Turkey

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Polycyclic aromatic hydrocarbons (PAHs) are produced during incomplete combustion of natural (e.g. coal, petroleum) or synthetic organic materials. Besides environmental exposure, occupational contact with PAHs at several work environments leads to high body burdens among the exposed workers. Epidemiologic studies have proved that exposure to PAHs increases the risk of cancer in the lungs, stomach, bladder and skin (IARC 1987). Exposure assessment is a critical step for the quantitative risk assessment of PAHs. As PAHs contain complex mixtures of more than 100 different compounds in the vicinity of coke oven areas, it is not feasible to evaluate each hazard. Monitoring a biological metabolite of a constant chemical in coke environment gives a correct risk assessment of PAHs (Wu et al. 1998).

1-hydroxypyrene (1-OHP), a metabolite of the non-carcinogenic PAH pyrene, provides more accurate assessment of total PAH exposure from all exposure routes, including dermal absorption, than PAH levels in air (Van Rooij JG et al. 1993). Increased urinary levels of 1-OHP have been measured in several environmental settings: coke plant workers, aluminum workers, road paving workers (Alexandrie et al. 2000; Carstensen et al. 1999; Burgaz et al. 1992; Burgaz et al. 1998), as well as air pollution (Merlo F e al, 1998). It is found that coke oven workers are at high risk of exposure to PAHs (Wu et al. 1998; Kuljukka et al. 2002). However, very limited data exist to date concerning the exposure of Turkish coke oven workers (Ates et al. 2004). The objective of the present study is to evaluate the exposure to PAHs among Turkish coke oven workers and the need for improvement of their work environment to reduce the risk of this chemical hazard. The present study is a part of comprehensive evaluation of health risk for PAH exposure in Turkish coke plant workers.

## MATERIALS AND METHODS

The coke oven group consisted of 50 male workers employed in a Turkish iron and steel plant in Eregli, Zonguldak. Eight of these workers were top-side workers (tar chaser, lidman, larry car operator) and 42 of them were side oven workers (wharfman, quencher, door repairman, benchman, pusher, door repairman,

*Correspondence to:* M. Yilmazer, 1005 Bryant Way, Suite #N, Sunnyvale, CA 94087, USA

**Table 1.** Demographic characteristics of the study population

| Variables | Controls | Coke Oven Workers |
|---|---|---|
| n | 42 | 50 |
| *Age* (mean±S.D.)[a] (range) | 37.86±9.77 (22-53) | 40.42±6.62 (25-49) |
| *Duration. of Exposure* (years) (mean±S.D.) | - | 13.84±6.80 |
| *Smoking*[b] | | |
| Non-smokers (n) | 7 (16.7%) | 18 (36.0%) |
| Smokers (n)[c] | 35 (83.3%) | 32 (64.0%) |
| 1-10 cig/day | 10 (23.8%) | 10 (20.0%) |
| 11-20 cig/day | 21 (50.0%) | 19 (38.0%) |
| >20 cig/day | 4 (9.5%) | 3 (6.0%) |
| *Body Mass Index* (kg/m$^2$) (mean±S.D.)[d] | 25.7±3.02 | 26.7±3.60 |

[a,d] $p>0.05$, [b] $X^2=3.39$, $p=0.066$, [c] $X^2=0.075$, $p=0.78$

supervisor, temperature controller, heater, body repairman). A control group consisted of 42 male individuals from the same plant employed at packaging and other works. All of the workers had working clothes, helmet, shoes, gloves and masks during their work. All participants in the study provided their informed consents. The questionnaire contained items about demography, work history, job description, protective measures, smoking status, dietary information (alcohol consumption, fruit, grilled meat, vitamins, etc.), heating types at homes, and medication (past and present). Characteristics of the studied population are summarized in Table 1. Persons worked less than 3 months and undergoing medical treatment, radiology or vaccination up to 3 months before sampling were not included in the study. All subjects had mixed diet. The postshift urine samples from the workers were collected in a PVC container without preservatives in spring and kept at -20°C until analysis.

Urinary 1-OHP was determined by an adaptation of the method of Jongeneelen et al. (1987). Twenty ml of 0.1 M acetate buffer (pH 5.0) was added into an aliquot of 10 ml of urine and then adjusted to pH 5.0 with 4.0 N hydrochloric acid. This mixture was incubated for 16 h with 12.5 $\mu$l of $\beta$-glucuronidase/sulfatase at 37°C in an electronically controlled rotary shaking bath for an enzymatic hydrolysis of the conjugates. For the extraction of the PAH metabolites in urine, a purification cartridge with $C_{18}$ reversed-phase liquid chromatographic material was used in the next step. The cartridge was primed with 5 ml of methanol, followed by 5 ml of distilled water, and then the hydrolyzed sample was passed through the cartridge. Subsequently, the cartridge was washed with 10 ml distilled water. The retained solutes were eluted with 9 ml of methanol. The elute was evaporated to dryness

**Table 2.** Mean ±S.D levels of 1-OHP (μmol/mol creatinine) in urine of study population by smoking habits

| | Controls | Coke Oven Workers | | |
|---|---|---|---|---|
| | | Overall | Top Side | Side Oven |
| Overall | 0.31±0.25<br>(42)[a]<br>[0.01-1.29][b] | 1.68±2.39<br>(50)<br>[0.05-14.99] | 3.59±4.84<br>(8)<br>[0.40-14.99] | 1.31±1.41<br>(42)<br>[0.05-7.63] |
| Smoking | | | | |
| Non-smokers | 0.28±0.23<br>(7)<br>[0.12-0.79] | 1.13±1.15<br>(18)<br>[0.05-3.77] | 1.61±1.82<br>(3)<br>[0.40-3.70] | 1.03±1.03<br>(15)<br>[0.05-3.77] |
| Smokers | 0.31±0.26<br>(35)<br>[0.01-1.29] | 1.99±2.83<br>(32)<br>[0.37-14.99] | 4.78±5.89<br>(5)<br>[0.47-14.99] | 1.47±1.57<br>(27)<br>[0.37-7.63] |

[a](n); [b][Range]

at 40°C under nitrogen gas. The residue was dissolved in methanol and run in HPLC with fluorescence detection that had excitation and emission wavelengths adjusted to 242 nm and 388 nm, respectively. 25 μl of the extracted sample was injected to a 150 x 4.6 mm inside diameter Lichrosorb RP18 (5 μm) column. The column temperature and the flow rate of mobile phase were 40°C and 0.8 ml/min, respectively. 1-OHP was eluted with methanol and water. The initial 57% methanol was not changed for 5 min, and it was increased to 69% over 20 min, and stayed same for 2 min, and then turned to initial conditions with a linear gradient function. 1-OHP gave a peak at 17.0 min. Urinary 1-OHP concentrations were corrected for creatinine concentrations and were expressed as μmol/mol creatinine.

Data were analyzed using SPSS version 10, 1999. For categorical data, Chi-square tests were performed to compare groups. Student *t* test, analysis of variance (ANOVA) were used to test significant differences among groups. *p*-values $<0.05$ were considered significant.

## RESULTS AND DISCUSSION

Table 1 indicated that both groups were well matched with regard to their age, smoking habits and intensity. The number of smokers was higher in the control group, but the difference was not significant. The urinary 1-OHP levels were significantly higher in coke oven workers (1.68±2.39 μmol/mol creatinine) than in controls (0.31±0.25 μmol/mol creatinine) ($p<0.001$). As shown in Table 2, the urinary 1-OHP concentrations of top side (3.59±4.84 μmol/mol creatinine) and side oven (1.31±1.41 μmol/mol creatinine) of coke oven workers were also significantly higher than that of controls, ($p<0.001$ and $p<0.01$, respectively).

**Table 3.** Some previous studies of 1-hydroxypyrene in urine of coke oven workers in different countries

| Mean 1-Hydroxypyrene (μmol/mol creatinine) (n) | Country | Reference |
|---|---|---|
| 2.30 (56) | The Netherlands | Jongeneelen, 1992 |
| 0.39 (33) | Belgium | Van Hummelen et al., 1993 |
| 25.00 (80) | Taiwan | Wu et al., 1998 |
| 1.00 (76) | Italy | Brescia et al., 1999 |
| 8.70 (21) | Estonia | Kuljukka et al., 2002 |
| 10.90 (37) | China | Hanaoka et al., 2002 |
| 9.00 (50)* | Poland | Siwinska et al.,2004 |
| 1.80 (32)* | Germany | Rihs et al., 2005 |
| **1.68 (50)** | **Turkey** | **This study** |

*Median

Top side oven workers also had significantly higher urinary 1-OHP levels than side oven workers ($p<0.001$). In this study, the concentration of urinary 1-OHP of coke oven workers was 5.4 times higher than that of controls.

There was an increase in urinary 1-OHP concentrations of smokers in coke oven workers, but this was not significant compared to those of non-smoker workers ($p>0.05$) (Table 2). Our results were in agreement with other studies such as coke oven workers (Zhang et al. 2001), iron foundry workers (Alexandrie et al. 2000; Carstensen et al. 1999), and asphalt workers (Burgaz et al. 1998). It is known that the influence of smoking on 1-OHP excretion in high exposure levels to PAHs like coke oven workers was minimal (Buchet et al. 1992). However, our results were contrary to Jongeneelen et al. (1990)'s study. These investigators found high levels of urinary 1-OHP concentrations in non-smoker coke oven workers which might be an explanation of this inconsistency. It was also contrary to Ates et al. 2004's study that revealed smoking as a considerable factor in PAH exposure evaluation. However, total number of workers in that sudy was 13, 9 of whom were smokers.

We also found that the urinary mean 1-OHP level of non-smokers of coke oven workers (1.13±1.15 μmol/mol creatinine) was significantly higher than that of smokers (0.31±0.26 μmol/mol creatinine) ($p<0.001$) and that of non-smokers in controls (0.28±0.23 μmol/mol creatinine) ($p<0.01$) indicating that the occupational exposure to PAHs was more than the exposure to PAHs by smoking. In this study, there was not a significant effect of smoking on urinary 1-OHP levels in controls. Similar results have recently been reported also by other researchers (Burgaz et al. 1998; Zhang et al. 2001). In contrast, Carstensen et al. (1999) and Alexandrie et al. (2000), have found a significant effect of smoking on urinary 1-OHP concentrations.

The concentration of urinary 1-OHP of control group in our study was lower than those of Chinese controls (Hanaoka et al. 2002; Leng et al. 2004) and higher than

that of Swedish, Italian and Korean controls (Alexandrie et al. 2000; Brescia et al. 1999; Carstensen et al. 1999; Yang et al. 2003). The discrepancy observed among the studies could be due to the distinct life style factors of the study populations such as smoking habits, passive smoking, diet, and heating with coke at homes. The urinary 1-OHP levels of smokers (0.31±0.26 μmol/mol creatinine) and non-smokers (0.28±0.23 μmol/mol creatinine) in control group were very similar to the urinary 1-OHP levels of smokers (0.33±0.19 μmol/mol creatinine) and non-smokers (0.24±0.13 μmol/mol creatinine) of the control group of a previous study carried out by Burgaz et al. (1992) in the Turkish population.

Several research groups have studied the internal PAH exposure in coke plants located in different countries since 1990. The results are shown in Table 3 in comparison with those of our study. Studies performed in west European coke oven workers revealed lower levels of 1-OHP (less than 3 μmol/mol creatinine) which were in line with our results. However, the internal exposure to PAHs of Polish, Chinese, Estonian and Taiwanese coke oven workers were about 3-8 folds higher than those of aforementioned studies, possibly due to the old technical standards and/or improper individual protective measures. An earlier study estimated that a urinary 1-OHP level of 2.3μmol/mol creatinine (Occupational exposure limit-OEL) corresponded to a relative risk of lung cancer of ~ 1.3 (Jongeneelen 1992), and the levels in 16 % of Turkish coke workers in the plant exceeded this risk level. Thus, it seems that the working conditions in this Turkish coke oven plant still need to be improved and it stresses the necessity for an individual determination of internal PAH exposure for health surveillance.

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