BIOMARKERS, 2000, VOL. 5, NO. 5, 368–381

Urinary excretion of 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons from different sources

MARINA BURATTI[1*], ORONZO PELLEGRINO[1]
GABRI BRAMBILLA[2] and ANTONIO COLOMBI[2]

[1] Istituti Clinici di Pefezionamento, Laboratorio di Tossicologia Professionale, Via S. Barnaba 8, I-20122 Milan, Italy

[2] University of Milano, Department of Occupational Health, Via S. Barnaba 8, I-20122 Milan, Italy

Received 17 November 1999, revised form accepted 28 April 2000

1-Hydroxypyrene (1-OHP) urinary excretion has been studied in subjects exposed to polycyclic aromatic hydrocarbons (PAH) from different sources (urban air pollution, cigarette smoking, food contamination or occupational exposure). In Study A, statistically significant differences among subjects categorized according to daily cigarette consumption were observed: 1-OHP median excretion of heavy smokers (more than 20 cigarettes per day; 1-OHP = 371 ng l^{-1}; $n = 6$) was significantly increased over that of non-smokers (1-OHP = 160 ng l^{-1}; $n = 79$), light smokers (less than 10 cigarettes per day, 1-OHP = 157 ng l^{-1}; $n = 7$) and also medium smokers (10–20 cigarettes per day, 1-OHP = 154 ng l^{-1}; $n = 13$) ($p < 0.04$). In smokers, 1-OHP excretion (y, ng l^{-1}) increased with the intensity of cigarette consumption and was associated with self-reported number of cigarettes smoked daily (x, n) ($y = 20+16.6x$; $r = 0.58$, $n = 22$, $p<0.01$), urinary thiocyanate (x, μmol l^{-1}) ($y = 55+2.6x$; $r = 0.57$, $n = 20$, $p<0.01$) and cotinine (x, μg l^{-1}) ($y = 89+0.23x$; $r = 0.62$, $n = 17$, $p<0.01$). In Study B the influence of smoked food consumption on 1-OHP excretion was evaluated: 1-OHP excretion began to increase as soon as 3 h after a PAH-rich meal and peak values were reached between 6 and 9 h after lunch. Maximum excretion mean values were respectively 525 ng l^{-1} for non-smokers ($n = 8$) and 650 ng l^{-1} for smokers ($n = 4$). 1-OHP concentrations in next-morning samples were back to pre-lunch levels both for non-smokers and smokers. In Study C non-smoker workers ($n = 28$) occupationally exposed to PAH in a steel plant were investigated. At values of airborne pyrene ranging between 6 and 30 μg m^{-3}, excretion values of 1-OHP up to 80 000 ng l^{-1} were observed. The use of urinary 1-OHP as a screening test to discriminate between smokers and non-smokers in the presence of uncorrected dietary influence has been calculated according to a cut-off value of 461 ng l^{-1} (reference group upper limit): the 1-OHP positive predictive value is 57%, its predictive negative value is 77%, sensitivity is 15% and specificity is 96%. In conclusion, 1-OHP appears to be a valuable biomarker of pyrene exposure. It will be nevertheless more accurate in assessing human PAH exposure from multiple sources if the influence of different kinetics for inhaled (particulate or gaseous) or ingested PAH are considered and if the role of oxidative polymorphism is adequately elucidated. The possibility of using 1-OHP to estimate the total burden of PAH from different sources or of screening groups with different PAH exposure appears to be a possible approach. However, the use of 1-OHP to evaluate the associated risk of cancer is still a premature target.

Keywords: polycyclic aromatic hydrocarbons, biological monitoring, urinary 1-hydroxypyrene, environmental exposure, occupational exposure, tobacco smoking, diet.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large family of ubiquitous organic compounds containing only carbon and hydrogen atoms, whose molecular

* Corresponding author: Marina Buratti, Istituti Clinici di Pefezionamento, Laboratorio di Tossicologia Professionale, Via S. Barnaba 8, I-20122 Milan, Italy. e-mail: marina.buratti@unimi.it

Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 2000 Taylo
http://www.tandf.co.uk/journals

structure is made up of three or more condensed benzene rings. Thermodynamically-stable PAHs are commonly formed during pyrolysis and incomplete combustion of organic materials, for example in the baking or frying of food such as meat, or from tobacco combustion. Large quantities of PAHs are also released in the environment from power generation plants, residential heating, vehicle fuel combustion and industrial emissions. Uptake into the organism may occur by inhalation, ingestion and dermal absorption. PAH exposure occurs in the general population as a consequence of urban air pollution, tobacco smoking, consumption of smoked or grilled food, and use of coal tar-based ointment medications. Occupational exposure to PAHs occurs during coke production by low-temperature coking of coal, in petrochemical cracking processes, in graphite electrode production, in foundries and during road construction (IARC 1986, Sithisarankul *et al*. 1997).

Assessment of exposure could be carried out by environmental monitoring of airborne PAH concentration, but sources other than inhalation are disregarded when this method is used. A better estimate of total PAH dose entering the organism, taking into account different absorption routes, could be obtained through the measurement of PAH metabolites excreted in urine (IPCS 1993). However, the measurement of urinary PAH metabolites is difficult to interpret for many reasons. Environmental and dietary PAHs of toxicological interest are complex mixtures of more than a dozen different chemicals, each of which is metabolized to several compounds. Only a fraction of PAH metabolites is eliminated in the urine, since, for instance, sulphate, glucuronyl or glutathione conjugates are excreted mainly in the faeces. Therefore, in low-level exposure such as the one occurring in dietary exposure, urinary concentrations of PAH metabolites are in the ng l^{-1} range and extremely sensitive analytical methods are required for their quantification. To overcome these limitations which are always present in biological monitoring, many different urinary PAH metabolites have been proposed as biomarkers for the assessment of exposure: among them, 1-hydroxypyrene (1-OHP), the major metabolite of pyrene excreted in urine, is generally the preferred tracer (Bouchard and Viau 1999). Pyrene is a four-ring compound quantitatively contributing to environmental or dietary PAH mixtures. Its concentration has demonstrated good correlation with total PAH and also with benzo[*a*]pyrene, one of the most potent and widespread human carcinogens (Kuljukka *et al*. 1996). Urinary 1-OHP concentration has been found to be positively associated with total PAHs in different airborne or alimentary exposures and over a wide range of PAH doses. Increased mean levels of 1-OHP, calculated on a group basis, have been reported in human biomonitoring studies on workers occupationally exposed to coal tar fumes, to mineral oil bitumen, or after tobacco smoking, and grilled or smoked food consumption in the general population. Reported data on the relationship between PAH exposure and 1-OHP show some discrepancies among different authors, mainly related to a large inter/intraindividual variability: many interfering factors, including age and gender, genetic polymorphism, pre-existing diseases or uncontrolled factors, such as diet, non-essential habits (cigarette smoking, alcohol consumption, medicine consumption), or concomitant chemical exposures, may contribute to this variability.

In this paper we present the results of two studies conducted to evaluate the influence of lifestyle habits and diet (tobacco smoking and smoked food consumption) on 1-OHP excretion values in subjects exposed to urban air

contamination (Studies A and B). The use of the 1-OHP measurement as a screening test in discriminating smokers and non-smokers was also evaluated. For the sake of comparison, 1-OHP results observed in the biological monitoring of PAH exposure in workers from a steel plant are also reported (Study C). Lastly, the usefulness of this biomarker to assess PAH exposure and to estimate health risks is discussed.

Materials and methods

Description of study populations

Study A: influence of smoking habit and of urban airborne pollution. The studied population consisted of 105 apparently healthy male subjects environmentally exposed to urban air pollution in a large town of northern Italy: 62 were police officers engaged as traffic wardens in streets in the town centre; 43 were police officers working as white-collar employees, doing clerical activities in an indoor office environment. Subjects were classified into four subgroups according to cigarette consumption: non-smokers ($n = 79$); light smokers smoking no more than 10 cigarettes per day ($n = 7$); medium smokers smoking between 10 and 20 cigarettes per day ($n = 13$); heavy smokers smoking more than 20 cigarettes per day ($n = 6$). Information on cigarette consumption and on anamnestic variables (age, alcohol, medicine consumption, etc.) were recorded by questionnaire. Spot urine samples were obtained at 12:30 h, before the lunch interval (noon sample). As soon as possible after urine collection, 1-ml aliquots were separated and stored in polyethylene tubes at –18 °C until analysis.

Inhalation exposure to airborne PAHs was assessed on 5-h personal air samples, collected from personal active impactors worn in the respiratory zone by 34 subjects (21 traffic wardens, 13 white-collar workers) from the beginning of the workshift at 7:30 h to before the lunch break at 12:30 h,. Time weighted average concentrations of pyrene, benzo*[a]*pyrene and total polycyclic aromatic hydrocarbons (tPAH, obtained from the sum of anthracene, fluoranthene, pyrene, crysene, benzo*[k]*fluoranthene, benzo*[b]*fluoranthene and benzo*[a]*pyrene selected as tracer compounds because of their constant presence among the 15 PAHs looked for in air samples) were then calculated.

Study B: influence of PAH-rich meal. The effect of consumption of a PAH-rich meal was investigated by studying the kinetics of excretion of 1-OHP in spot urine samples obtained by 13 volunteers (9 non-smokers, 4 smokers). Subjects participating in the experiment consumed PAH-rich food ad libitum (smoked ham and salmon, smoked cheese, wood-oven baked bread and pizza) during a banquet, held between 12:00 and 14:00 h. Spot urine samples were collected 2 h before lunch, and from all voids for the 24 h post-meal. As soon as possible after urine collection, 1-ml urine aliquots were separated and stored in polyethylene tubes at –18 °C until analysis. A total number of 129 spot urine samples were analysed for 1-OHP and creatinine concentrations. No attempts were made to measure the content of PAHs in the different dishes or to measure the individual quantity of ingested food.

Study C: biological monitoring of occupationally-exposed workers. Occupationally-exposed non-smoker male workers employed at an Italian steel plant were monitored ($n = 28$): 20 of them were coke oven workers and eight worked at the steel blast furnace. They were daily entrusted with 11 different duties, all dealing with the processing of raw materials and steel, including coal, coke, iron ore, molten iron and molten steel. Information about the usual individual confounding factors (age, alcohol consumption, medicine assumption, etc.) were recorded by an anamnestic questionnaire. Personal protective equipment was available in some jobs, but the continuity of its use was difficult to confirm by questionnaire. Subjects were requested to restrain from consumption of grilled/roasted, broiled or smoked food in the 72 h preceding and during urine sample collection. Spot urine samples for 1-OHP analysis were obtained from all workers at least once at the end of an 8-h workshift. Furthermore, 20 subjects repeated the collection of end-of-shift urine samples for three non-consecutive working days. As soon as possible after collection, 1-ml urine aliquots were separated and stored in polyethylene tubes at –18 °C until analysis.

Environmental monitoring of airborne PAH pollution was done by 8-h area monitoring carried out by stationary sampling at three positions chosen as being representative of PAH exposure because of the prolonged presence of workers in those locations. The area sampling was performed for two workshifts, concurrently to urine collection.

Assay of urinary 1-hydroxypyrene

1-OHP in urine was quantitated according to a modified HPLC method, modelled after that of Jongenleen and modified in our laboratory (Jongenleen *et al.* 1987). Enzymatic hydrolysis of the

glucuronide and sulphate 1-OHP conjugates was accomplished with β-glucuronidase/sulphatase, type H-2, from *Helix pomatia* (Sigma, Milan, Italy). Urine samples (1 ml), diluted with 1 ml of acetate buffer (0.2 M, pH 5.0), containing 250 U of β-glucuronidase and 20 U of sulphatase, were incubated for 16 h at 37°C. Samples were then purified by extraction with 3 ml of ethyl acetate; the organic phase (2.5 ml), was transferred into a glass vial and taken to dryness at 40 °C under a stream of nitrogen. The residue was dissolved with 200 µl of mobile phase and 40 µl-aliquots of the resulting solution were injected into chromatograph. The analyses were performed with a Waters HPLC Module 1plus (Waters Italia, Vimodrone, Italy) equipped with a fluorescence detector (Perkin Elmer LS-1) (Perkin Elmer Italia, Monza, Italy). The wavelengths used for quantification were 340 nm for excitation and 390 nm for emission. Chromatographic separation was obtained by isocratic elution at 35 °C on a reverse-phase Supelcosil-DP column (50 mm × 4.6 mm i.d., 5-µm filling), coupled with a Supelguard DP precolumn (20 mm × 4.6 mm i.d.; 5 µm filling) (Supelco-Aldrich, Milan, Italy). The composition of the mobile phase was: acetonitrile–water–acetic acid 30:69.5:0.5; the flow was 4 ml min^{-1}. Retention time for 1-OHP was 4.1 min and the entire run required 5 min. The limit of detection of the method was 50 ng l^{-1}; day-to-day imprecision, calculated at concentration of 1-OHP of 385 ng l^{-1}, was Cv<10 % ($n=12$). Calibration was linear in a range from 50 to 5000 ng l^{-1}; samples showing 1-OHP values higher than the upper linearity limit were properly diluted and reinjected. The mean recovery of the analyte, determined in spiked urine samples at three concentrations (500, 5000 and 25 000 ng l^{-1}), was 88 ± 5 % ($n=10$). Quality performances of the analytical procedure were assessed by repeated analysis of ClinChek Urine Controls (Recipe Chemicals, Munich, Germany). The values of 1-OHP obtained were respectively 1.8 ± 0.1 mcg l^{-1} and 8.1 ± 0.2 mcg l^{-1} (mean and standard deviation, 5 determinations for each level), in good accordance with certified 1-OHP concentrations ($1\text{-OHP}_{\text{level I}} = 1.8$ mcg l^{-1}, range: 1.0-2.6, and $1\text{-OHP}_{\text{level II}} = 8.2$ mcg l^{-1}, range: 5.4–11.0).

Other methods

PAH environmental air analysis was performed by personal and area samplers; adsorbents were eluted with CH_2Cl_2 and analysed by HPLC with fluorometric detection (NIOSH 1985).

Urinary creatinine was determined photometrically through a Hitachi 917 analyser. Urinary cotinine was determined through an in-house modified HPLC method (Pichini *et al*. 1991). Urinary thiocyanate was determined according to a highly-sensitive and specific colorimetric method (Buratti *et al*. 1997).

Statistical analysis

Statistical analyses were performed using Statgraphics-plus software package version 3 (Statistical Graphics Corporation, Rockville, Maryland, USA). Data were analysed by non-parametric statistics, not assuming normal distribution of data. Kruskal–Wallis and Mann–Whitney W tests were used to compare medians; the Kolmogorov–Smirnov test was run to compare distributions, and Spearman rank correlation coefficients were determined to study associations between variables. Differences among groups were considered statistically significant when the p-value was below 0.05. For statistical elaboration, non-detectable concentrations of 1-OHP were assigned a value of 25 ng l^{-1}, one-half of the limit of detection.

Results

Study A: influence of urban airborne pollution

The exposure to airborne PAH pollution during a 5-h workshift in an urban environment was measured by environmental and biological monitoring, 5-h time weighted average concentrations of airborne PAHs (values expressed in ng m^{-3}), are reported in table 1. Statistically significant associations were found between airborne tPAH (y, ng m^{-3}) and pyrene values (x, ng m^{-3}) ($y = 0.2 + 4.6x$, $r = 0.88$, $n = 34$, $p < 0.01$), tPAH (y, ng m^{-3}) and benzo[*a*]pyrene (x, ng m^{-3}) ($y = 1.4 + 5.9x$, $r = 0.83$, $n = 34$, $p < 0.01$), and pyrene (y, ng m^{-3}) vs benzo[*a*]pyrene (x, ng m^{-3}) ($y = 0.5 + 1.0x$, $r = 0.76$, $n = 34$, $p < 0.01$).

Comparison of tPAH, pyrene and BaP median values from subjects dichotomously classified according to outdoor vs indoor job environment (street or office) failed to show any statistical difference between traffic wardens and white-collar workers; smoking habits of monitored subjects did not appear to influence values of airborne PAH sampling to any extent.

Table 1. Influence of urban air PAH pollution on 1-hydroxypyrene urinary excretion (Study A). Values of airborne polycyclic aromatic hydrocarbons (expressed in ng m^3) are calculated as 5-h time weighted averages from personal passive samplers; urinary concentration of 1-hydroxypyrene, in spot samples collected after 5-h workshift, are expressed in ng l^{-1} and ng g^{-1} creatinine.

	Traffic wardens					White-collar workers				
				1-hydroxypyrene					1-hydroxypyrene	
	[a]tPAH (ng m^{-3})	Pyrene (ng m^{-3})	[b]BaP (ng m^{-3})	(ng l^{-1})	(ng g^{-1} creatinine)	[a]tPAH (ng m^{-3})	Pyrene (ng m^{-3})	[b]BaP (ng m^{-3})	(ng l^{-1})	(ng g^{-1} creatinine)
Mean	6.6	1.3	0.9	239	147	5.9	1.6	0.7	217	125
SD	3.5	0.6	0.5	312	174	2.9	1.0	0.4	209	86
Median	5.1	1.1	0.9	184	114	4.4	1.2	0.5	157	109
Min.	4.2	0.8	0.5	<50[c]	ne	2.6	0.4	0.1	<50[c]	ne
Max.	16.4	3.5	2.5	1971	1037	10.1	4.2	1.3	1032	348
n	21	21	21	62	62	13	13	13	43	43

[a] tPaH values were calculated as sum of concentrations of six most representative polycyclic aromatic hydrocarbons (anthracene, fluoranthene, pyrene, crysene, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene and benzo[*a*]pyrene).

[b] BaP = benzo[*a*]pyrene.

ne = not evaluable.

SD = standard deviation; min. = minimum value; max. = maximum value; *n* = number of subjects.

[c] Analytical detection limit.

Urinary concentrations of 1-OHP, measured in spot urine samples collected at the end of a 5-h workshift, are also reported in table 1: values are expressed in ng l^{-1} and ng g^{-1} creatinine.

Comparison of 1-OHP urinary excretion values between traffic wardens and white-collar workers, performed with values expressed both as ng l^{-1} and ng g^{-1} creatinine, showed no statistical difference between groups. Moreover, in people ($n = 35$) subjected to environmental and biological monitoring of exposure, airborne PAH urban air pollution and urinary 1-OHP individual excretion values were not related.

Study A: influence of smoking habit

To assess the effect of smoking habits on 1-OHP excretion, results from the whole cohort of Study A were pooled, because there was no difference between traffic wardens and white-collar workers. Urinary 1-OHP values observed in Study A subjects, categorized according to smoking habit (smokers or non-smokers) or to the number of cigarettes consumed daily (non-smokers, light-, medium- or heavy-smokers) are reported in table 2: values are in ng l^{-1}. We have chosen not to adjust 1-OHP values by urinary creatinine concentration since this had little effect on the results of our analyses or our conclusions. Looking at the distribution of all 1-OHP values observed, detectable amounts of 1-OHP were found only in 80% of urine samples (85 out of 105 subjects), while the presence of results below the analytical detection limit (50 ng l^{-1}) was equally distributed in non-smokers and smokers (13/79 vs 4/26). Thus, even among smokers, certain subjects excrete undetectable amounts of 1-OHP.

When 1-OHP median results were compared between groups of non-smokers and smokers, the increase in urinary 1-OHP concentrations in smokers did not reach any statistical significance. However statistically significant differences were

Table 2. Urinary concentration of 1-hydroxypyrene (1-OHP, ng l^{-1}), thiocyanate (SCN, μmol l^{-1}) and cotinine (COT, μg l^{-1}), observed in urine samples from Study A subjects classified according to cigarette consumption level as: non-smokers, light smokers (< 10 cigarettes per day), medium smokers (10–20 cigarettes per day), and heavy smokers (> 20 cigarettes per day).

	Non-smokers			Light smokers			Medium smokers			Heavy smokers			All smokers		
	1-OHP (ng l^{-1})	SCN (μmol l^{-1})	COT (μg l^{-1})	1-OHP (ng l^{-1})	SCN (μmol l^{-1})	COT (μg l^{-1})	1-OHP (ng l^{-1})	SCN (μmol l^{-1})	COT (μg l^{-1})	1-OHP (ng l^{-1})	SCN (μmol l^{-1})	COT (μg l^{-1})	1-OHP (ng l^{-1})	SCN (μmol l^{-1})	COT (μg l^{-1})
Mean	213	24	–	157	56	629	257	88	990	508	180	1749	281	105	1136
SD	271	8	–	97	22	318	309	31	709	312	63	761	280	61	772
Median	160	24	–	157	48	611	154	91	875	371	195	1744	187	92	984
Min.	<50	9	–	<50[b]	33	251	<50[b]	40	210	186	88	883	<50	33	<50
Max.	1971	42	<50[a]	315	92	1087	1032	145	2831	962	275	3236	1032	275	210
n	79	72	55	7	6	7	13	13	8	6	6	9	26	25	21

[a] All subjects had values below 50 μg l^{-1}, limit of detection of the analytical method.
[b] Analytical detection limit.

found among subjects categorized according to daily cigarette consumption. 1-OHP median excretion of heavy smokers (more than 20 cigarettes per day) was significantly increased over that of non-smokers, light smokers (less than 10 cigarettes per day) and also medium smokers (10–20 cigarettes per day) ($p < 0.04$). No differences were found among medium smokers, light smokers or non-smokers. In smokers, values of 1-OHP excretion (y, ng l^{-1}) increased with the intensity of cigarette consumption and resulted associated with self-reported daily smoked cigarette number (x, n) ($y = -17 + 14.5x$; $r = 0.65$, $n = 22$, $p < 0.01$). Table 2 also reports the values of urinary thiocyanate and cotinine, two well-accepted biomarkers of smoking habits, which were obtained in the groups of subjects investigated, the groups being classified according to cigarette consumption level. Both thiocyanate and cotinine differ between smokers and non-smokers and their median values increase in accordance with increased cigarette consumption. 1-OHP excretion values were associated and increased either with values of thiocyanate (x, μmol l^{-1}) ($y = 46 + 2.5x$; $r = 0.58$, $n = 20$, $p < 0.01$) or cotinine (x, μg l^{-1}) ($y = 89 + 0.23x$; $r = 0.75$, $n = 19$, $p < 0.01$).

The compliance between 1-OHP–cotinine–thiocyanate and self-reported absence of cigarette consumption has been verified. Among subjects with cotinine and thiocyanate values respectively below 50 μg l^{-1} and 100 μmol l^{-1} (true non-smokers), 1-OHP values ranging from < 50 ng l^{-1} to 1971 ng l^{-1} were observed. Setting the upper limit for the non-smoker reference group at 461 ng l^{-1} (a value corresponding to the 95th percentile calculated from the distribution of values), three outlier subjects are observed, with excretion values of 1-OHP respectively of 588, 1391 and 1971 ng l^{-1}.

Study B: influence of PAH-rich meal on 1-OHP urinary excretion levels

Kinetics of 1-OHP urinary excretion observed in subjects after a banquet rich in smoked food, plotted according to smoking habit of subjects (non-smokers ✳, smokers o), are illustrated in figure 1. 1-OHP concentration values (ng l^{-1}) found in individual spot samples are plotted vs time of urination (h), starting from lunchtime at 14:00 h, set as 0 h, and followed for 24 h. Pre-lunch 1-OHP values are different between smokers and non-smokers and are in the range of values observed in subjects of Study A, either for non-smokers and smokers. After the meal – as soon as 3 h later – 1-OHP excretion began to increase and maximum values were reached between 6 and 9 h after lunch. Maximum values at the time of maximum excretion were respectively 525 ng l^{-1} for non-smokers and 650 ng l^{-1} for smokers. 1-OHP concentrations in next-morning samples were back to the pre-lunch level both for non-smokers and smokers. A food-related mean increase of 1-OHP excretion of about 150 ng l^{-1}, calculated as the mean of differences between paired maximum and pre-lunch individual values, has been observed both for non-smoker and smoker groups.

Study C: influence of airborne PAH occupational exposure on 1-OHP urinary excretion levels

Environmental monitoring of PAH pollution in coke oven workers showed a wide range of airborne contamination with variable values of total PAHs (sum of 15 PAHs) ranging from 2 μg m^{-3} to 185 μg m^{-3}, depending on sampling location in the plant.

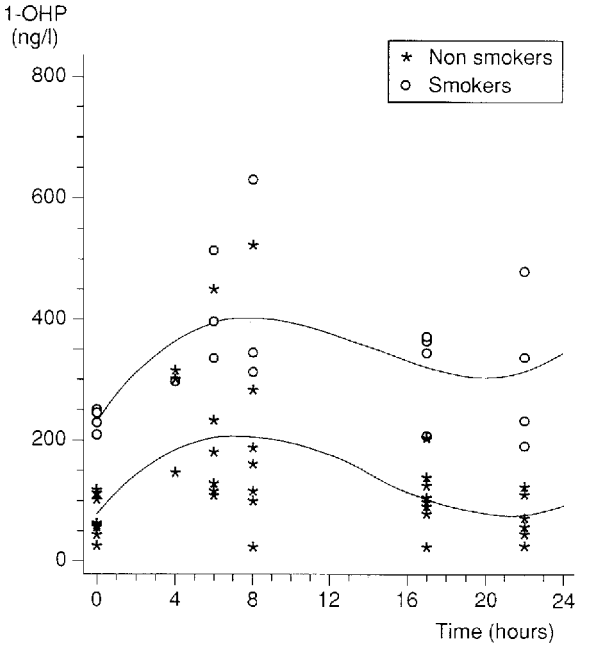

Figure 1. Patterns of 1-hydroxypyrene urinary excretion (1-OHP, ng l^{-1}) in non-smokers (*) and smokers (o) after a PAH-rich meal. Lunchtime is defined as 0 h; collection time was computed as hours elapsed from lunchtime.

Because of the many work tasks in which workers were involved and the limited extension of the studied group, jobs have been tentatively classified on the basis of the time spent and the physical location in which workers were stationed during the performance of their assigned jobs (Costantino *et al*. 1995). Three main job categories were defined, according to measured average PAH exposure, including: (a) workers who spent the entire workshift on the top of ovens or at blast furnace (battery operators, lidmen and steel production plant workers: exposure level-3; airborne pyrene = 6.5–36.4 μg m^{-3}), (b) workers mainly assigned at the side of ovens (coke side machine operators: exposure level-2; pyrene = 2.7–14.9 μg m^{-3}) and (c) workers with other job titles (exposure level-1; pyrene = 0.5–11.2 μg/m^3). 1-OHP urinary excretion values obtained in Study A were adopted as reference (exposure level-0). To compare 1-OHP excretion among referents and workers categorized as above detailed, individual values were averaged in the 20 subjects which gave spot samples over 3 days.

Results of biological monitoring of PAH occupationally exposed workers grouped according to job position, are summarized in figure 2. Post-shift values of 1-OHP excretion observed during the three non-consecutive days survey ranged from 890 ng l^{-1} to 82 500 ng l^{-1} and were always higher than values observed in Study A for non-smokers (upper limit for non-smoker reference group: 461 ng l^{-1}).

From results reported in figure 2, it appeared that level-3 workers received the highest levels of exposure to PAH, showing highest levels of 1-OHP excretion. Level-2 workers showed 1-OHP medium excretion values and level-1 workers had in general lowest values. Results of comparisons among exposure levels showed statistically significant differences either between referents or exposed workers and

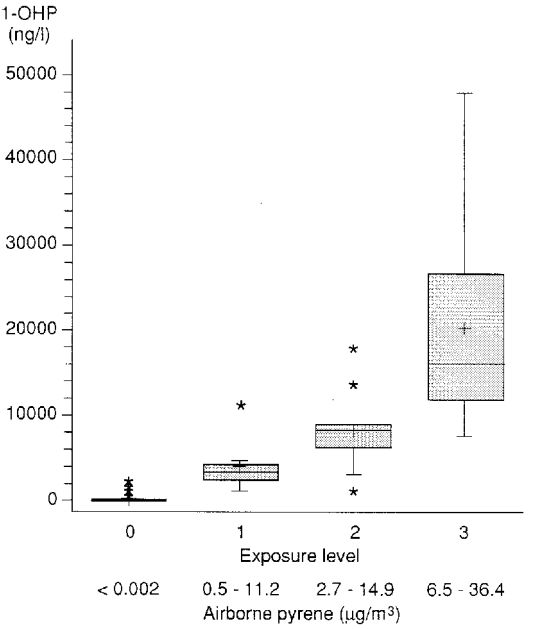

Figure 2. Concentrations of 1-hydroxypyrene (1-OHP, ng l^{-1}) determined in urine samples of Study A (exposure level = 0) and Study C steel plant workers, classified according to job categories as low-exposed to airborne pyrene (pyrene between 0.5 and 11.2 µg m^{-3}; exposure level = 1), medium-exposed (pyrene between 2.7 and 14.9 µg m^{-3}; exposure level = 2) and high-exposed (pyrene between 6.5 and 364 µg m^{-3}; exposure level = 3). The means were calculated from the individual urine values of n subjects who each gave three spot samples over the 3-day survey. Exposure level-1, $n = 7$; level-2, $n = 4$; level-3, $n = 9$. The plotted data are divided into four areas of frequency. The box extends from the lower quartile to the upper quartile, covering the centre half of each sample. The horizontal line inside the box represents the median. The whiskers extend from the box to the minimum and maximum value of each sample, except for outside points (which lie more than 1.5 times the interquartile range above or below the box), which are plotted separately (*). Mean values are indicated by the + symbol.

among the three categories, with 1-OHP median values respectively of 3375 ng l^{-1} (level-1, $n = 7$), 8837 ng l^{-1} (level-2, $n = 4$), and 17 007 ng l^{-1} (level-3, $n = 9$).

The wide variability in 1-OHP excretion here observed either in samples from the same subject or among different people entitled of similar job tasks, could at least in part be explained by irregular use of respiratory protective equipment.

Predictivity of the test for smoking habit

Results from Study A were used to assess the performance of 1-OHP urinary excretion in classifying subjects (all with uncontrolled dietary habits) with respect to self-declared smoking habit, urinary cotinine and thiocyanate excretion. The predictivity of urinary 1-OHP as a screening test to discriminate between smokers and non-smokers in presence of uncorrected dietary influence has been calculated according to a cut-off value set at 461 ng l^{-1} (95th percentile of non-smoker distribution). The 1-OHP positive predictive value (VP+ = $4 \times 100/7$, percent of true smokers among people who were positive to 1-OHP test, because of 1-OHP values higher than 461 ng l^{-1}) is 57%, and the predictive negative value

(VN− = 76 × 100/98, percent of true non-smokers among all those who were negative to the 1-OHP test) is 78 %, with a sensitivity, Se, of 15 % (Se = 4 × 100/26, percent of smokers correctly detected among all smokers) and a specificity, Sp, of 96 % (Sp = 76 × 100/79, percent of non-smokers correctly detected among all non-smokers). No significant increase in predictive values has been observed, when the smoking habit of subjects was classified according to their cotinine or thiocyanate urinary excretion (cut-off values respectively of 100 $\mu g\,l^{-1}$ and 100 $\mu mol\,l^{-1}$).

Discussion

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 PAH of different origins (Viau *et al*. 1999).

The main object of this paper was to evaluate the adequacy of urinary 1-OHP assay as a biomarker for assessment of PAH exposure in different exposure levels, routes and sources of exposure such as urban air pollution, smoking habit, PAH-rich food consumption or PAH occupational exposure.

Results of environmental and biological monitoring observed in subjects exposed to urban PAH pollution (Studies A and B) show low levels of exposure, with values quite comparable with those from other studies on similarly exposed groups. Differences in PAH intake reported by different authors, excluding relevant analytical influence or inadequate sampling methods which could invalidate the comparison, are related to the different dietary habits of investigated subjects (Buckley and Lioy 1992, Zhao *et al*. 1992, Van Rooij *et al*. 1994, Viau *et al*. 1995, Gilbert and Viau 1997).

In Study A the trivial influence of environmental PAH exposure confirmed the relevance of other sources of PAH exposure on 1-OHP excretion in the groups considered (table 1). In fact an uptake of about 4 ng of pyrene inhaled during the 5 h monitored period (calculated from a airborne pyrene mean concentration = 2 $ng\,m^{-3}$, and considering that normal breathing corresponds approximately to inhalation of 0.6 $m^3\,h^{-1}$) could not give rise to an appreciable increase of excretion in comparison with baseline urinary values in the order of hundreds of $ng\,l^{-1}$. It is thus possible that urinary 1-OHP excretion is poorly influenced by low-level PAH exposure such as that corresponding to total airborne PAH concentrations below 10 $ng\,m^{-3}$ occurring as a consequence of urban air pollution.

The relevant role of tobacco smoking on 1-OHP excretion was confirmed by the positive and significant association observed in smokers between 1-OHP values and self-reported cigarette consumption ($r = 0.58$, $p < 0.01$, $n = 22$). This association is also confirmed by other urinary biomarkers of smoking habit (cotinine $r = 0.62$, and thiocyanate $r = 0.57$). As observed by other authors, about 40 % of intrasubject variability in 1-OHP excretion values could be related to smoking habit (Van Rooij *et al*. 1994, Roggi *et al*. 1997). Otherwise it could also be underlined that, on a group basis, consumption of up to 20 cigarettes per day did not significantly contribute to 1-OHP excretion, while consumption of more than 20 cigarettes per day doubled 1-OHP values when compared with moderate smokers and non-smokers: 1-OHP median urinary concentrations measured in our study were respectively 305 and 157 $ng\,l^{-1}$ for heavy smokers and non-smokers (table 2).

According to the literature, main stream smoke of a cigarette contains 50–270 ng pyrene and the contribution of cigarette smoking to pyrene daily intake is in the range 1–10 mcg per day for a moderate habit (IARC 1986). Assuming in the present study a daily pyrene intake through inhaled cigarette smoke of about 2000 ng (calculated from an average amount of pyrene delivered to the lung from one cigarette of 100 ng, and a consumption of 20 cigarettes per day) only 10% of inhaled dose appears in urine as 1-OHP. The limited influence of moderate smoking on 1-OHP excretion is confirmed by the low predictive value (VP+) of 1-OHP excretion as a screening test for smoking habit. In subjects on an unrestricted diet only 57% of positive subjects (those having 1-OHP excretion values higher than 461 ng l^{-1}, cut-off value corresponding to 95° percentile of reference distribution) are true smokers. With regard to the apparently good specificity of the test (Sp = 96%), it should be pointed out that among the 105 studied subjects, only four smokers and three non-smokers had excretions higher than the cut-off value. The limited number of cases considered should be taken into account in relation to the Sp value obtained.

Also food contributes significantly to 1-OHP excretion. Previous reports of controlled feeding studies have shown strong association between urinary 1-OHP and recent consumption of charbroiled meat (Buckley and Lioy 1992, Kang *et al*. 1995, Sithisarankul *et al*. 1997). Our results from subjects consuming a PAH-rich meal (Study B) confirmed the influence of food on 1-OHP excretion. Increased urinary values were obtained and very similar kinetics of time-related excretion among different people were observed. The profile of 1-OHP excretion reported in figure 1 is in good accordance with the kinetic model proposed by Buckley (Buckley and Lioy 1992) which estimated a mean $t_{1/2}$ of 4.4 h and t_{max} of 6.3 h and also with the kinetics of urinary excretion of 1-OHP in rats after oral exposure (Viau *et al*. 1999). Excretion kinetics observed here in different subjects, while similar in shape, are associated with a wide range of individual 1-OHP values, reflecting the presence of large metabolic variability. The range of 1-OHP values observed at t_{max}, could be tentatively assigned to a genetic difference in pyrene metabolite production as suggested by results of more closely controlled PAH exposure (Göen *et al*. 1995, Hong *et al*. 1999). To this end, the steepness of the slope of individual excretion curves could be considered a marker of differences in individual metabolic activity (data not shown), as suggested by other authors (Grimmer *et al*. 1997, Merlo *et al*. 1998).

According to the 1-OHP excretion kinetic curve, increased 1-OHP urinary values in morning spot samples might be related to the amount of PAHs ingested with food at dinner the previous evening. The existence of such a source of PAH uptake could not be ruled out in Study B, because no restrictions had been set on consumption of PAH-containing food during the day preceding urine sample collection. A non-smoker was excluded from Study B, because he showed anomalous 1-OHP excretion at the beginning of the survey (values before, 6 h and 24 h after experimental PAH consumption were respectively 368, 330 and 52 ng l^{-1}). On enquiry, he confirmed the consumption of wood-oven baked pizza at lunch and dinner on the day preceding the experiment.

From all the collected data it could be stated that smoked food consumption and tobacco smoking habit (heavy consumption) play similar roles in increasing short-term 1-OHP excretion levels at least under the experimental conditions described in the present study. In fact an average increase in 1-OHP excretion of about

200 ng l^{-1} has been observed as a consequence of either a PAH-rich lunch (Study B) or smoking habit (Study A).

These observations confirm the necessity to consider separately groups of non-smokers and smokers and to acquire anamnestic data on PAH-rich food consumption when measuring 1-OHP, for non-occupational exposure surveys.

Finally, considering the interindividual variability of 1-OHP excretion, it is interesting to underline the presence also of subjects with undetectable excretion values (negative outliers). In non-occupationally-exposed people, comparable frequencies of negative outliers (subjects with values of 1-OHP below 50 ng l^{-1}) have been observed in non-smokers (13 out of 79) and true smokers (5 out of 25) (the status of active smoker being verified by the high urinary excretion of cotinine and thiocyanate). These findings still do not have a convincing explanation, but this repeatedly verified evidence, perhaps observed but not underlined by other authors (Boogard and van Sittert 1994, Göen *et al*. 1995, Viau *et al*. 1995) remain intriguing. From our results it seems that there are subjects with reduced capacity to absorb and/or eliminate pyrene from the body as urinary 1-OHP. In these subjects, urinary 1-OHP excretion does not correlate with external exposure, smoking habit or consumption of smoked food. Studies of some life-style habits or constitutive factors acting as determinants of variability suggest conflicting evidences of alcohol and smoke as microsomal enzyme inducers and possibly disclose the existence of a genetic polymorphism of oxidative PAH enzymes (Merlo *et al*. 1998, Gabbani *et al*. 1999, Hong *et al*. 1999).

In Study C a group of 28 workers employed in a steel production plant was investigated. Values of pyrene airborne concentration ranged between 0.5 and 40 μg m^{-3}, and excretions of 1-OHP up to 80 000 ng l^{-1} were observed, associated with both job category and estimated level of PAH exposure. Even in this case, the relationship between individual daily PAH exposure and 1-OHP excretion was fairly low and the influence of diet as an unaccounted factor of variation of urinary 1-OHP levels was negligible (Boogaard and van Sittert 1994, Kang *et al*. 1995, Dell'Omo *et al*. 1998, Wu *et al*. 1998a,b).

Results obtained here from the investigated groups can be briefly summarized as follows:

In the general population, cigarette smoking and/or diet accounted for over 90% of total pyrene intake while contributions from urban pollution are much lower and generally negligible (Van Rooij *et al*. 1994).

Heavy smokers show an appreciable increase of 1-OHP excretion on a group basis, but otherwise 1-OHP values poorly correlate with individual smoking habit (Zhao *et al*. 1992, Roggi *et al*. 1997, Sithisarankul *et al*. 1997).

Consumption of grilled or smoked food constitutes a relevant source of PAH exposure and appears to be a determinant of the same order of magnitude of smoking on 1-OHP excretion (Van Rooij *et al*. 1994).

After the same total PAH dose, there is a high interindividual variability of 1-OHP excretion, related mainly to uncontrolled (or unknown) sources of metabolic variance (Viau *et al*. 1999).

In subjects heavily exposed to PAHs in industrial environments, the contribution of smoking habit and/or diet is negligible (Wu *et al*. 1998a,b).

In conclusion, the high level of association between 1-OHP and total PAH reported in many studies and confirmed also by our results, suggests that 1-OHP is one of the most promising tracers for PAH exposure assessment. 1-OHP appears

sensitive enough to serve as an indicator of exposure to PAHs, provided that the fraction of pyrene in the PAH mixture of interest has been established. The composition of a PAH mixture from a given source is in fact relatively constant, but it could severely differ according to different origins. It is well known that PAHs from different sources show marked differences in benzo*[a]*pyrene concentration and in the ratio between pyrene and the carcingenic PAHs.

According to the previous statements, the possibility of using 1-OHP to estimate the total burden of PAH from different sources or to screen groups with different PAH exposure appears a reasonable approach. Nevertheless, before urinary 1-OHP could be used as dose biomarker of PAH exposure for assessment of cancer risks, deeper knowledge has to be acquired on the carcinogenicity of PAH mixtures.

References

Boogaard, P. J. and van Sittert, N. J. 1994, Exposure to polycyclic aromatic hydrocarbons in petrochemical industries by measurement of urinary 1-hydroxypyrene. *Occupational and Environmental Medicine*, **51**, 250–258.

Bouchard, M. and Viau C. 1999, Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: biological monitoring strategies and methodology for determining biological exposure indices for various work environments. *Biomarkers*, **4**, 159–187.

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.

Buratti, M., Xaiz, D., Caravelli, G. and Colombi, A. 1997, Validation of urinary thiocyanate as biomarkers of tobacco smoking. *Biomarkers*, **2**, 81–85.

Costantino, J. P., Redmond, C. K. and Bearden, A. 1995, Occupationally related cancer risk among coke oven workers: 30 years of follow-up. *Journal of Occupational and Environmental Medicine*, **37**, 597–604.

Dell'Omo, M., Muzi, G., Marchionna, G., Latini, L., Carrieri, P., Paolemili, P. and Abbritti, G. 1998, Preventive measures reduce exposure to polycyclic aromatic hydrocarbons at graphite plant. *Occupational and Environmental Medicine*, **55**, 401–406.

Gabbani, G., pavanello, S., Nardini, B., Tognato, O., Bordin, A., Fornasa, C. V., Bezze, G. and Clonfero, E. 1999, Influence of metabolic genotype GSTM1 on levels of urinary mutagens in patients teated topically with coal tar. *Mutation Research, Genetic Toxicity & Environmental Mutagenesis*, **440**, 27–33.

Gilbert, N. L. and Viau, C. 1997, Biological monitoring of environmental exposure to PAHs in the vicinity of Söderberg aluminium reduction plant. *Occupational and Environmental Medicine*, **54**, 619–621.

Göen, T., Gündel, J., Schaller, K. H. and Angerer, J. 1995, The elimination of 1-hydroxypyrene in the urine of the general population and workers with different occupational exposure to PAH. *Science of the Total Environment*, **163**, 195–201.

Grimmer, G., Jacob, J., Dettbarn, G. and Naujack, K. W. 1997, 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.

Hong, Y. C., Leern, J. H., Park, H. S., Lee, K. H., Lee, S. J., Lee, C. K. and Kang, D. H. 1999, Variations in urinary 1-hydroxypyrene glucuronide in relation to smoking and the modification effects of GSTM1 and GSTT1. *Toxicology Letters*, **108**, 217–223.

Jongenleen, F. J., Anzion, R. B. M. and Henderson, P. T. 1987, Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *Journal of Chromatography*, **412**, 227–232.

Kang, D. H., Rothman, N., Poirier, M. C., Greenberg, A., Hsu, C. H., Schwartz, B. S., Baser, M. E., Groopman, J. D., Weston, A. and Strickland, P. T. 1995, Interindividual differences in the concentration of 1-hydroxypyrene-glucuronide in urine and polycyclic aromatic hydrocarbon–DNA adducts in peripheral white blood cells after charbroiled beef consumption. *Carcinogenesis*, **16**, 1079–1085.

Kuljukka, T., Vaaranrinta, R., Veidebaum, T., Sorsa, M. and Peltonen, K. 1996, Exposure to PAH compounds among cokery workers in the oil shale industry. *Environmental Health Perspectives*, **104** (suppl. 3), 539–541.

IARC 1986, Evaluation of carcinogenic risk of chemicals in human: tobacco smoking. *IARC Monograph 38* (Lyon: International Agency for Research on Cancer).

IPCS 1993, Biomarkers and risk assessment. Concepts and principles. *Environmental Health Criteria 155* (Geneva: International Programme on Chemical Safety, World Health Organization).

MERLO, F., ANDREASSEN, A., WESTON, A., PAN, C. F., HAUGEN, A., VALERIO, F., REGGIARDO, G., FONTANA, V., GARTE, S., PUNTONI, R. and ABBONDANDOLO, A. 1998, Urinary excretion of 1-hydroxypyrene as a marker for exposure to urban air levels of polycyclic aromatic hydrocarbons. *Cancer Epidemiology, Biomarkers & Prevention,* **7**, 147–155.

NIOSH 1985, *Polynuclear Aromatic Hydrocarbons. Method 5506-1* (Cincinnati, OH: National Institute for Occupational Safety and Health).

PICHINI, S., ALTICHIERI, I., PACIFICI, R., ROSA, M. and ZUCCARO, P. 1991, Elimination of caffeine interference in high-performance liquid chromatographic determination of cotinine in human plasma. *Journal of Chromatography (Biomedical Applications)*, **586**, 267–269.

ROGGI, C., MINOIA, C., SCIARRA, G. F., APOSTOLI, P., MACCARINI, L., MAGNAGHI, S., CENNI, A., FONTE, A., NIDASIO, G. F. and MICOLI, G. 1997, Urinary 1-hydroxypyrene as marker of exposure to pyrene: an epidemiological survey on a general population group. *Science of the Total Environment*, **199**, 247–254.

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

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 sources of internidividual variability in the baseline excretion of 1-hydroxypyrene in urine. *International Archives of Occupational and Environmental Health*, **66**, 55–65.

VIAU, C., VYSKOČIL, A. and MARTEL, L. 1995, Background urinary 1-hydroxypyrene levels in non-occupationally exposed individuals in the Province of Québec, Canada, and comparison with its excretion in workers exposed to PAH mixtures. *Science of the Total Environment*, **163**, 191–194.

VIAU, C., BOUCHARD, M., CARRIER, G., BRUNET, R. and KRISHNAN, K. 1999, The toxicokinetics of pyrene and its metabolites in rats. *Toxicology Letters*, **108**, 201–207.

WU, M. T., MAO, I. F., HO, C. K., WYPIJ, D., LU, P. L., SMITH, T. J., CHEN, M. L. and CHRISTIANI, D.C. 1998a), Urinary 1-hydroxypyrene concentrations in coke oven workers.*Occupational and Environmental Medicine*, **55**, 461–467.

WU, M. T., WYPIJ, D., HO, C. K., MAO, I. F., CHEN, M. L., LU, P. L. and CHRISTIANI, D. C. 1998b), Temporal changes in urinary 1-hydroxypyrene concentrations in coke-oven workers. *Cancer Epidemiology, Biomarkers & Prevention*, **7**, 169–173.

ZHAO, Z. H., QUAN, 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 polycyclic aromatic hydrocarbons. *Science of the Total Environment*, **113**, 197–207.