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# Urinary hydroxylated metabolites of polycyclic aromatic hydrocarbons as biomarkers of exposure in asphalt workers

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**Abstract**

**Background.** Fumes and vapours released during laying of hot asphalt mix have been recognised as a major source of exposure for asphalt workers. **Objectives**. We investigated the relationships between inhalation exposure to asphalt emissions and urinary biomarkers of polycyclic aromatic hydrocarbons (PAHs) in asphalt workers (AW, $n=75$) and in ground construction workers (CW, $n=37$). **Methods**. Total polyaromatic compounds (PAC) and 15 priority PAHs in inhaled air were measured by personal sampling. Hydroxylated PAH metabolites (OH-PAHs) (2-naphthol, 2-hydroxyfluorene, 3-hydroxyphenanthrene, 1-hydroxypyrene, 6-hydroxychrysene and 3-hydroxybenzo[a]pyrene) were determined in urine spot samples collected in three different times during the work week. **Results**. Median vapour-phase PAC (5.5 $\mu g\ m^{-3}$), PAHs ($\leq 50$ ng $m^{-3}$) and OH-PAHs (0.08–1.11 $\mu g\ l^{-1}$) were significantly higher in AW than in CW, except in the cases of air naphthalene and 2-naphthol. Airborne levels of particle-phase contaminants were similar in the two groups and much lower than vapour-phase levels; metabolites of particulate PAHs were never found in quantifiable amounts. An appreciable increase in OH-PAH levels during the work day and work week was found in AW; median levels for 2-hydroxyfluorene, 3-hydroxyphenanthrene and 1-hydroxypyrene were, respectively, 0.29, 0.08 and 0.18 at baseline; 0.50, 0.18 and 0.29, pre-shift; 1.11, 0.44 and 0.44 $\mu g\ l^{-1}$, post-shift. Each OH-PAH exhibited a characteristic profile of increase, reflecting differences in half-lives of the parent compounds. In non-smoking subjects, positive correlations were found between vapour-phase PAC or PAHs and OH-PAHs both in pre- and post-shift samples ($0.34 \leq r \leq 69$). Smokers exhibited 2–5-fold higher OH-PAHs than non-smokers, at any time and at both workplaces. **Conclusions**. Our results suggest that OH-PAHs are useful biomarkers for monitoring exposure to asphalt emissions. The work-related exposure to PAC and PAHs was low in all AW, but urinary metabolites reflected exposure satisfactorily.

**Keywords:** *Bitumen emissions, polycyclic aromatic hydrocarbons, urinary metabolites, asphalt pavers, biological monitoring, biomarker*

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## Introduction

Asphalt (called bitumen in Europe) is a dark, semi-solid material, occurring naturally or produced by non-destructive distillation of crude oil during petroleum refining. For binding properties, asphalt is mixed with inorganic fillers (sand, gravel, limestone) and various additives to form asphalt mix used in road paving (European Oil Company Organisation for Environment, Health and Safety (CONCAWE) 1992). When asphalt products are applied at elevated temperatures, fumes and vapours are released. Emissions contain a multitude of organic chemicals: aliphatic compounds (the dominant compounds), cyclic alkanes, aromatic hydrocarbons and polyaromatic compounds (PAC). The PAC family encompasses a wide variety of congeners, such as aromatic derivatives with various substituent groups, i.e. alkyl, hydroxy, carbonyl, nitro or thio groups, heterocyclic compounds containing oxygen, sulphur and nitrogen atoms, and polycyclic aromatic hydrocarbons (PAHs) (Hicks 1995, CONCAWE 2002, Heikkila et al. 2002, International Programme on Chemical Safety (IPCS) 1998, 2004). Some volatile and semi-volatile PAC may have irritative effects; moreover, a few PAC have been recognised as mutagenic and possibly carcinogenic (Jacob 1980, International Agency for Research on Cancer (IARC) 1985, 1987, 2002, IPCS 2004). Individuals employed in the road construction industry are potentially exposed to asphalt emissions either during asphalt mixing or road paving through inhalation of fumes and vapours, and via skin contact (Burstyn 2001, National Institute of Occupational Safety and Health (NIOSH) 2001, Boffetta et al. 2003, Brandt & Watson 2003). Inhalation exposure of asphalt workers is usually evaluated by measuring PAC and PAHs in workplace air, with special attention paid to particle-phase PAHs (CONCAWE 2002, NIOSH 1998a,b). However, there is also a need for a comprehensive measure of the actual amount absorbed into the body by other means, such as dermal and gastric routes. Biological monitoring is the technique of choice for this purpose, as it gives an estimate of the internal dose by measurement of biomarkers (the parent compound or a metabolite) in biological tissues and fluids. To date, biomarkers specific to asphalt emission exposure have not been identified. For a long time, 1-hydroxypyrene (1OHP), a urinary metabolite of pyrene, has been used as unique biomarker of exposure to PAHs from any source (Hatjian et al. 1995, Bouchard & Viau 1999, McClean et al. 2004a). However, pyrene is only one PAH out of several hundreds and it may not be the most relevant marker to represent PAH exposure in all instances. As an integrative measure of exposure to complex mixtures of PAHs, urinalysis of multiple PAH metabolites has been carried out recently in different occupational settings (Strunk et al. 2002, Elovaara et al. 2003, Carmella et al. 2004, Kim et al. 2001, Väänänen et al. 2005, 2006).

This communication is a partial report of a comprehensive study in which biological, dermal and ambient exposure measurements were made to assess levels and routes of exposure to asphalt emissions among a group of asphalt workers (AW) engaged in asphalt mixing and road paving in comparison with a group of ground construction workers (CW), chosen as controls. A full description of the study design and other results of environmental and biological monitoring have been published earlier (Cirla et al. 2005, Campo et al. 2006a,b).

Here we focus on excretion of mono hydroxy derivatives of naphthalene (2-naphthol), fluorene (2-hydroxyfluorene), phenanthrene (3-hydroxyphenanthrene), pyrene (1-hydroxypyrene), chrysene (6-hydroxychrysene) and benzo[a]pyrene (3-hydroxybenzo[a]pyrene), determined in spot urine samples collected at three different

time points during the work week. In addition, the relationships between the indicators of contamination of the workplace air and biomarkers are reported and discussed in AW as compared to CW. In this framework, the influence of tobacco smoking on concentrations of urinary hydroxylated metabolites (OH-PAHs) and the general feasibility of the use of these parameters in the occupational setting were also assessed.

## Materials and methods

### *Study design*

The survey was conducted in spring/summer 2003 on workers engaged in road construction in urban, suburban and rural areas of the Milan and Lodi provinces in Italy (Cirla et al. 2005, Campo et al. 2006a,b). The weather was warm (16–32°C) and clear. Although 147 subjects were enrolled in the overall project, a total of 112 subjects (75 AW and 37 CW) were available for this investigation, consisting entirely of Caucasian men aged 22–75 years (mean 43 years). Their length of employment at the firm averaged 11 years (1–46 years) and they were all in good health clinically. AW (43 non-smokers, 32 smokers), exposed to asphalt emissions during mixing or laying of hot asphalt mix, were employed in different tasks, described by the following job titles: seven hot-mix asphalt workers, eight truck drivers, eight paver operators, 42 rakermen, ten roller drivers. The asphalt mix contained 5–6% petroleum-based bitumen; the range of application temperatures was 120–260°C (median value 130°C). The paving machines were not equipped with a cabin or a ventilation system. Workers did not wear respiratory masks or use any barrier creams. Because of warm weather, workers were usually wearing shorts and T-shirts, but they were provided with protective gloves, shoes and high-visibility equipment. Working clothes were water-washed weekly. The management did not provide the workers with underwear or socks, or laundering facilities for these items. Other hygienic aids (provision of clean work clothes, showering facilities) were rarely available at workplaces.

CW (12 non-smokers, 25 smokers), employed in ground constructions and not working with hot-mix asphalt, were selected as the 'unexposed to asphalt emissions' reference group. Both groups were exposed to vehicle exhausts from working machines powered by diesel engines and, occasionally, from surrounding vehicular movement.

To reduce the possible confounding effect of diet on biological monitoring, all subjects were required to refrain from intake of PAH-rich food (i.e. smoked and grilled food, wood-oven cooked pizza, tea, coffee, etc.) 12 h prior and during the day of sampling. Before sampling, each subject was interviewed by an occupational health physician who filled in a questionnaire with information about lifestyle, diet, medical history and occupational activity. Written informed consent was obtained from each subject prior to the study.

### *Assessment of exposure to airborne contamination*

The day of sampling took place on the third or fourth shift of subsequent workdays. To allow determination of asphalt-emission exposure, workers were fitted with personal monitoring devices, worn in the respiratory zone during the first part of a work shift (typically 07.00–11.00). Air samples were collected in accordance with NIOSH

method 5506 (NIOSH 1998b). The air sampling system consisted of a Teflon filter (37-mm diameter, 2-μm pore size, Zefluor filter, Supelco, Milan, Italy) to collect particulate material, backed with a XAD-2 sorbent tube (ORBO 42 LG, Supelco) containing 100 and 50 mg XAD-2 resin in the front and back sections, respectively, to collect vapour-phase PAC. Air was sampled at a nominal flow rate of 2 l $min^{-1}$, for an average sampling duration of 4 h (range 3–7 h). Samples, preserved from degradation by sunlight, were transported in coolers and refrigerated at 2–8°C until extraction, which was performed within 48 h of sample receipt. Gravimetric analysis for total particulate matter collected on filters was conducted according to NIOSH method 0500 (NIOSH 1994).

### *Analysis of airborne PAC and PAHs*

We measured the concentrations of PAC and 15 PAHs (naphthalene (NAP), acenaphthene (ACN), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), chrysene (CHR), benzo[a]anthracene (BAA), benzo[k]fluoranthene (BKF), benzo[b]fluoranthene (BBF), benzo[a]pyrene (BAP), dibenzo[a,h]anthracene (DBA), benzo[g,h,i]perylene (BPE) and indeno[1,2,3-cd]pyrene (IPY)) separately in the vapour- and the particle-phase samples.

### *Sample preparation*

Membrane filters and XAD-adsorbents, transferred to 4-ml glass vials with Teflon-lined caps, were extracted with acetonitrile (2 ml) by sonication for 30 min at 40°C. Particulate extracts (P-PAC including PYR, CHR, BAA, BKF, BBF, BAP, DBA, BPE, IPY) and vapour-phase extracts (V-PAC including NAP, ACN, FLE, PHE, ANT, FLT, PYR) were stored separately at 2–8°C until instrumental analysis.

### *Determination of PAC by UV spectrophotometry*

An in-house UV spectrophotometric method has been developed as a screening tool to provide a relative assessment of the total PAC content of asphalt emissions. Because the toxicological properties of the chemicals present in the two phases are inherently diverse, and also because of their expected different bioavailability, PAC concentrations were determined separately in the two fractions. Briefly, an aliquot of the acetonitrile extract was transferred to a 1-cm path length quartz cell and its absorbance at 254 nm ($UV_{254}$) was recorded. The masses of V-PAC and P-PAC in unknown samples were determined with the help of concurrently prepared calibration graphs, constructed by plotting the $UV_{254}$ ($y$, abs) versus known concentrations of PHE or BKF solutions ($x$, μg $ml^{-1}$), chosen as representative of the vapour- and particulate-phase PAC, respectively. The choice of calibrators was based on a number of facts. Molar absorption of PHE at 254 nm is one of the strongest among the considered volatile PAHs: it is about 30 times higher than that of 2-ring PAHs, like NAP, and more than three times that of FLU and PYR. PHE was always found in all our samples, and its concentration was second only to NAP. Finally, the chemico-physical properties of PHE fall midway between those of 2- and 4-ring PAHs, and, thus, this feature also makes it a reasonable candidate to represent both families of congeners. Analogous arguments supported the choice of BKF as the reference compound for quantification of P-PAC mass. Obviously, the values obtained are an approximate estimate of the true

absolute amount of PAC. The analytical limit of detection was 0.03 μg $m^{-3}$ (corresponding to an airborne concentration of about 0.15 μg $m^{-3}$).

*Determination of specific PAHs by high-pressure liquid chromatography*

Once sample extracts had been analysed for PAC by UV spectrophotometry, they were subsequently analysed for individual PAH content as well. Separation and identification of single PAHs was afforded by high-pressure liquid chromatography (HPLC) with fluorescence detection, according to analytical conditions previously determined (Buratti et al. 2006).

*Urine sample collection*

We determined the concentrations of some representative OH-PAHs, namely 2-naphthol (2NAP), 2-hydroxyfluorene (2FLE), 3-hydroxyphenanthrene (3PHE), 1-hydroxypyrene (1OHP), 6-hydroxychysene (6CHR) and 3-hydroxybenzo[a]pyrene (3BAP) in replicate urine samples collected at different times across the work week. Three spot samples were obtained from each worker. The first one was collected on Monday at the beginning of the work week (around 07.00; second urination of the day; baseline). After two or three consecutive workdays, on the same day as the air sampling, two further urine samples were collected: prior (around 07.00, second urination of the day; pre-shift) and at the end of the shift (around 17.00; post-shift). Urine samples were stored at −20°C until analysis.



*Analysis of urinary hydroxylated PAH metabolites*

OH-PAHs are excreted in urine mainly as conjugates of glucuronic acid and sulfate. To release free OH-PAHs for analysis, urine samples (2 ml), buffered to pH 5 with 0.8 ml of 0.5 M acetate buffer containing 1000 U of β-glucuronidase and 80 U of sulphatase, were enzymatically deconjugated at 37°C overnight. Hydrolysed samples were extracted twice with a total of 4 ml diethyl ether; an aliquot of the organic phase (3 ml) was combined with 300 μl of alcoholic KOH (18 mM in methanol) and evaporated at 40°C under a stream of nitrogen. The recovery of all metabolites added in control urine was good (93–105%). A human OH-PAH-positive urine was run in each series as a quality control specimen. At the time of instrumental analysis, the residue was dissolved in 200 μl of mobile phase. Free OH-PAHs were simultaneously determined by HPLC with fluorimetric detection, according to a refinement of published methods (Gündel & Angerer 2000, Wang et al. 2005). A reverse-phase column (Supelcosil-DP column, 50 mm length, 4.6 mm internal diameter, 5 μm particle size) (Sigma Aldrich Supelco, Milan, Italy) was used for metabolite separation, using a binary gradient of an aqueous buffer (triethanolamine-HCl, pH 7.2, 10 mM, containing 0.8% n-butanol (BT) and acetonitrile (ACN)). During conditioning of the column and prior to injection, the mobile-phase composition was 82% BT and 18% ACT. After injection, the percentage of ACT increased according to a linear gradient as follows: 18% to 40% from 0 to 10 min; 40% from 10 to 13 min. After a 3-min period to re-equilibrate the column with the initial mobile phase, the system was ready for the next injection. The flow-rate was 2.4 ml $min^{-1}$, and the column temperature was stabilised at 35°C. The injection volume was 30 μl. The column eluent was monitored by automatic adjustment of the wavelength and

photomultiplier gain for each compound according to the retention time: 0–4 min, excitation and emission wavelengths 227 and 355 nm (2NAP); 4–7 min, 280/322 nm (2FLE); 7–8.2 min, 246/370 nm (3PHE); 8.2–10 min, 340/390 nm (1OHP); 10–11.8 min, 260/385 nm (6CHR); 11.8–13 min, 300/428 nm (3BAP). Quantitation was based on peak height and concentrations were calculated by external standardisation, using commercial reference compounds for calibration. The limits of detection were: 2NAP 0.2 $\mu g\, l^{-1}$; 2FLE and 3PHE 0.01 $\mu g\, l^{-1}$; 1OHP 0.02 $\mu g\, l^{-1}$; 6CHR and 3BAP 0.06 $\mu g\, l^{-1}$.

### *Determination of urinary cotinine*

Smoking habits of the studied subjects were objectified by analysing cotinine concentrations in pre-shift urine samples according to a HPLC method (Thuan et al. 1989). A cut-off value of 100 $\mu g\, l^{-1}$ of cotinine was applied for the subdivision of non-smokers ($\leq 100\ \mu g\, l^{-1}$) and smokers ($> 100\ \mu g\, l^{-1}$).

### *Adjustment for urinary creatinine*

Creatinine normalisation is a controversial issue and it should be used when metabolites are excreted primarily through renal filtration (Boeniger et al. 1993). Some authors have shown that creatinine adjustment can decrease the intra-individual source of variation, but at the same time it also tends to increase the inter-individual source of variation; therefore, on a group basis it may not always lead to statistical advantage (Symansky et al 2001, Viau et al. 2004). In fact, because many factors affect the rate of creatinine excretion (i.e. age, body mass index, physical activity, urine flow, time of the day and diet), creatinine adjustment, while correcting for dilution, introduces additional variation.

Taking all these factors into consideration, statistical tests were repeated with and without creatinine adjustment. Because only minor differences were observed with and without adjustment for creatinine, together with a general worsening of associations between environmental and creatinine-corrected biological indicators, only unadjusted levels of OH-PAHs are reported herein.

### *Statistical analysis*

Statgraphics statistical package (STSC, Rockville, MD, USA) was used for statistical elaborations. Environmental and biological data were log-transformed (natural), to best satisfy normal distribution assumption. Values below the detection limits were included in analyses as one-half the detection limit. Log-transformed data were compared using ANOVA, Student's *t*-test, paired *t*-test, and the Kolmogorov–Smirnov test. Pearson's correlations were used to test the associations between variables. Statistical significance is reported at the 0.05 level.

## Results

### *Environmental monitoring*

The levels of contaminants measured in breathing air were determined as indicators of work air contamination. A selection of the results (V-PAC, NAP, FLE, PHE and

Table I. Summary statistics of personal exposure to polycyclic aromatic compounds in asphalt (AW, $n=75$) and road construction workers (CW, $n=37$). The reported parameters are: polyaromatic compounds in vapour phase (V-PAC), and selected PAH concentrations. For each parameter mean and standard deviation (mean ± SD), median, minimum and maximum values (min, max) are given. See text for abbreviations.

| | V-PAC | NAP | FLE | PHE | PYR |
|---|---|---|---|---|---|
| Exposure group | ($\mu g\ m^{-3}$) | ($ng\ m^{-3}$) | ($ng\ m^{-3}$) | ($ng\ m^{-3}$) | ($ng\ m^{-3}$) |
| AW ($n=75$) | | | | | |
| mean ± SD | 6.7 ± 4.7 | 503 ± 368 | 48 ± 44 | 98 ± 147 | 47 ± 56 |
| median | 5.5 | 426 | 36 | 52 | 27 |
| (min, max) | (2.2, 35) | (84, 2139) | (<0.1*, 285) | (1, 1096) | (1.2, 282) |
| CW ($n=37$) | | | | | |
| mean ± SD | 4.8 ± 3.5 | 437 ± 232 | 18 ± 17 | 18 ± 11 | 1.4 ± 1.2 |
| median | 3.9† | 376 | 9† | 15† | 1.0† |
| (min, max) | (1.1, 16.1) | (113, 877) | (<0.1*, 53) | (2, 40) | (0.4, 4.9) |

*Limit of detection.
†$p$ <0.05 for $t$-test to compare exposure levels between AW and CW.

PYR), relevant to the scope of this paper, is presented in Table I. PAC concentrations are illustrated as an estimate of global contamination from the complex family of polyaromatic congeners (including PAHs non-alkylated, alkylated and substituted with various functional groups, and heterocyclic compounds) not otherwise achievable from PAH data. The partitioning of PAC was such that 80% was detected in the vapour phase (XAD tubes, V-PAC) and 20% in the particulate phase (filters, P-PAC). The V-PAC concentrations were low (range 1.1–35 $\mu g\ m^{-3}$), with a maximum value of about 35 $\mu g\ m^{-3}$ observed in AW. Inhalation exposure to V-PAC was higher among AW (5.5 $\mu g\ m^{-3}$) than among CW (3.9 $\mu g\ m^{-3}$). In contrast, P-PAC values were similar and low in both exposure groups (1.1 $\mu g\ m^{-3}$ in AW vs. 1.0 $\mu g\ m^{-3}$ in CW).

Concentrations of individual vapour-phase PAHs (NAP, ACN, FLE, PHE, ANT, FLT and PYR) ranged from <0.1 to 2300 $ng\ m^{-3}$. NAP was the most abundant compound. PYR was the only PAH present in both vapours and particulate. It was unevenly distributed between the two phases, the major part being found in the vapour-phase (80% in AW and 88% in CW). FLE, PHE and PYR significantly correlated with each other ($r=0.44$–$0.68$), and each of the three parameters correlated with V-PAC ($r=0.68$ for FLE; $r=0.76$ for PHE; $r=0.36$ for PYR). Levels of vapour-phase PAHs in AW were significantly higher than in CW, with the exception of NAP, whose airborne concentration was similar in the two exposure groups. Major differences were observed for FLE and PHE (AW values 4-fold higher than CW), and PYR (30-fold higher). Particle-phase PAHs were generally present in concentrations below 1 $ng\ m^{-3}$, and no differences between AW and CW were appreciable.

There was no statistical difference in exposure levels of airborne contamination for workers within the same exposure group when taking into account different job titles. Low total particulate matter values were observed and did not differ between exposure groups (median values 0.21 $mg\ m^{-3}$ in AW and 0.43 $mg\ m^{-3}$ in CW).

### *Biological monitoring*

Summary statistics of the results of biological monitoring in the study population, by exposure group and smoking status, are given in Table II. Measurable amounts of

Table II. Statistics of urinary excretion values of hydroxylated metabolites of volatile polycyclic aromatic hydrocarbons in asphalt (AW, $n = 75$) and road construction workers (CW, $n = 37$) grouped according to smoking status. The analytes studied are 2-naphthol (2NAP), 2-hydroxyfluorene (2FLE), 3-hydroxyphenanthrene (3PHE) and 1-hydroxypyrene (1OHP). For each metabolite three values are reported, observed, respectively, on Monday (baseline), before (pre-shift) and at the end (post-shift) of the shift after 2 or 3 consecutive workdays. For each parameter, the mean and standard deviation (mean ± SD) median value, minimum and maximum values (min, max) are given. All urinary values are expressed in $\mu g\ l^{-1}$.

| | 2NAP | 2FLE | 3PHE | 1OHP | 2NAP | 2FLE | 3PHE | 1OHP | 2NAP | 2FLE | 3PHE | 1OHP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | | | Pre-shift | | | | post-shift | | | |
| *Non-smoker* | | | | | | | | | | | | |
| AW ($n = 43$) | | | | | | | | | | | | |
| mean ± SD | 7.3 ± 5.7 | 0.45 ± 0.47 | 0.16 ± 0.26 | 0.28 ± 0.29 | 9.7 ± 9.0 | 0.69 ± 0.71 | 0.25 ± 0.30 | 0.51 ± 0.65 | 12.2 ± 15.8 | 1.45 ± 1.54 | 0.92 ± 2.02 | 0.68 ± 0.71 |
| median | 6.0 | 0.29 | 0.08 | 0.18 | 7.0 | 0.50 | 0.18 | 0.29 | 8.0 | 1.11 | 0.44 | 0.44 |
| (min, max) | (1.7, 31) | (0.1, 2.7) | (<0.01*, 1.48) | (<0.02*, 1.19) | (1.0, 54) | (0.1, 2.4) | (<0.01*, 0.92) | (<0.03*, 2.79) | (1.5, 91) | (0.03, 9.7) | (<0.01*, 8.4) | (<0.03*, 3.67) |
| CW ($n = 12$) | | | | | | | | | | | | |
| mean ± SD | 6.9 ± 3.7 | 0.22 ± 0.16 | 0.08 ± 0.11 | 0.13 ± 0.08 | 10.6 ± 10.9 | 0.36 ± 0.25 | 0.32 ± 0.84 | 0.18 ± 0.21 | 8.1 ± 4.6 | 1.08 ± 2.81 | 0.89 ± 2.25 | 0.23 ± 0.36 |
| median | 6.0 | 0.20 | 0.02† | 0.11† | 8.0 | 0.26 | 0.06† | 0.13† | 7.9 | 0.22† | 0.14† | 0.10† |
| (min, max) | (3.5, 15) | (0.1, 0.6) | (0.01, 0.38) | (0.02, 0.29) | (3.5, 42) | (0.1, 0.8) | (<0.01*, 3.3) | (0.02, 0.98) | (3.5, 20) | (0.1, 10) | (<0.01*, 8) | (0.06, 1.32) |
| *Smokers* | | | | | | | | | | | | |
| AW ($n = 32$) | | | | | | | | | | | | |
| mean ± SD | 13.3 ± 10.5 | 1.73 ± 1.05 | 0.37 ± 0.30 | 0.41 ± 0.30 | 18.3 ± 14.7 | 2.16 ± 1.38 | 0.75 ± 1.61 | 0.70 ± 0.44 | 19.9 ± 12.3 | 3.59 ± 2.05 | 1.08 ± 1.31 | 0.98 ± 1.06 |
| median | 10.5 | 1.35 | 0.32 | 0.33 | 13.3 | 1.90 | 0.36 | 0.62 | 16.5 | 2.90 | 0.68 | 0.64 |
| (min, max) | (2, 42) | (0.2, 3.5) | (<0.01*, 1.1) | (0.05,0.92) | (3, 68) | (0.2, 6) | (0.08, 10.1) | (0.11, 1.86) | (4, 56) | (0.7, 9.8) | (0.12, 7.3) | (0.20, 5.94) |
| CW ($n = 25$) | | | | | | | | | | | | |
| mean ± SD | 16.8 ± 16.1 | 1.88 ± 1.16 | 0.37 ± 0.33 | 0.48 ± 0.32 | 17.2 ± 16.7 | 2.20 ± 2.49 | 0.71 ± 2.00 | 0.55 ± 0.49 | 12.7 ± 6.8 | 2.66 ± 1.98 | 1.04 ± 2.88 | 0.59 ± 0.56 |
| median | 12.8 | 1.80 | 0.30 | 0.44 | 13.8 | 1.50† | 0.27 | 0.37† | 12.3† | 2.3† | 0.47† | 0.39† |
| (min, max) | (3.5, 80) | (0.1, 4.1) | (<0.01*, 1.59) | (0.06, 1.44) | (4.5, 90) | (0.1, 11) | (<0.01*, 11) | (0.08, 2.10) | (4.5, 40) | (0.1, 6) | (<0.01*, 14.5) | (0.08, 2.41) |

*Limit of detection.

†$p < 0.05$ for $t$-test to compare biomarkers levels between AW and CW.

hydroxylated metabolites of vapour-phase PAHs were found in the large majority of the urine samples. In fact, 2NAP and 2FLE were quantified in all samples, while 3PHE and 1OHP were found to be below the limits of detection in four samples (two AW and two CW), out of 112 studied subjects. Metabolites of particle-phase PAHs (6CHR and 3BAP) were always below the analytical limit of detection (0.06 $\mu g\, l^{-1}$). A description of the results is given below.

*Asphalt-exposed worker.* In non-smoking AW, the lowest concentrations of OH-PAHs were found in baseline urine samples collected on Monday morning, after over 60 h off work. Marked increases in excretion of 2FLE, 3PHE and 1OHP were observed both during the work shift and the work week, as shown, respectively, by the statistically significant differences evidenced between pre- and post-shift values, and between baseline and pre-shift values (paired-samples analysis). Net increases of excretion of 2FLE and 3PHE occurring during the work shift were significantly higher than those found between pre-shift and baseline samples (2.5-fold vs. 1.5-fold; *t*-test for paired samples). In the case of 1OHP, post-shift values 1.6-fold higher than pre-shift values and pre-shift values 1.5-fold higher than baseline values were observed. Significant increases in excretion of 2NAP could be observed during the work week (baseline vs. pre-shift samples); however, the increase during the work day was marginal ($p < 0.09$). Plots of the distributions of concentration values of the vapour-phase PAH metabolites in urine samples of non-smoking AW, collected at different sampling times during the work week, are presented in Figure 1, in which the different graphs relate to: (A) 2NAP, (B) 2FLE, (C) 3PHE and (D) 1OHP. No differences were found in OH-PAH excretion values of non-smoking AW grouped according to job titles.

Current smoking increased the metabolite levels by a factor of 2–5 in comparison to non-smokers. A significant gradual rise of excretion values was also confirmed in smokers both during the work shift and through the work week. Comparison of data for non-smoking and smoking AW showed parallel behaviour: the net differences between the urinary concentrations of smokers at the different time points were comparable with those of non-smokers.

*Ground construction workers.* In non-smoking CW, levels of OH-PAHs were similar at any sampling time point. A significant trend towards increasing values over both the work shift and the work week was observed only for 3PHE. Similar to findings in AW, increased levels of all the metabolites were found in CW smokers compared with non-smokers.

*Comparison between AW and CW.* The following findings refer to non-smokers, unless otherwise stated. The data summarised in Table II indicate that concentrations of OH-PAHs in AW were higher than in CW at any time point, apart from 2NAP whose excretion levels were always similar in the two exposure groups. In general, results differed substantially between exposure groups, with the exception of 2FLE in baseline ($p < 0.07$) and pre-shift samples ($p < 0.10$). In this case, the threshold of significance was not reached, probably because of the small number of CW. In smokers, the statistical significance of the comparisons among the variables in the two exposure groups confirmed the results obtained in non-smokers.

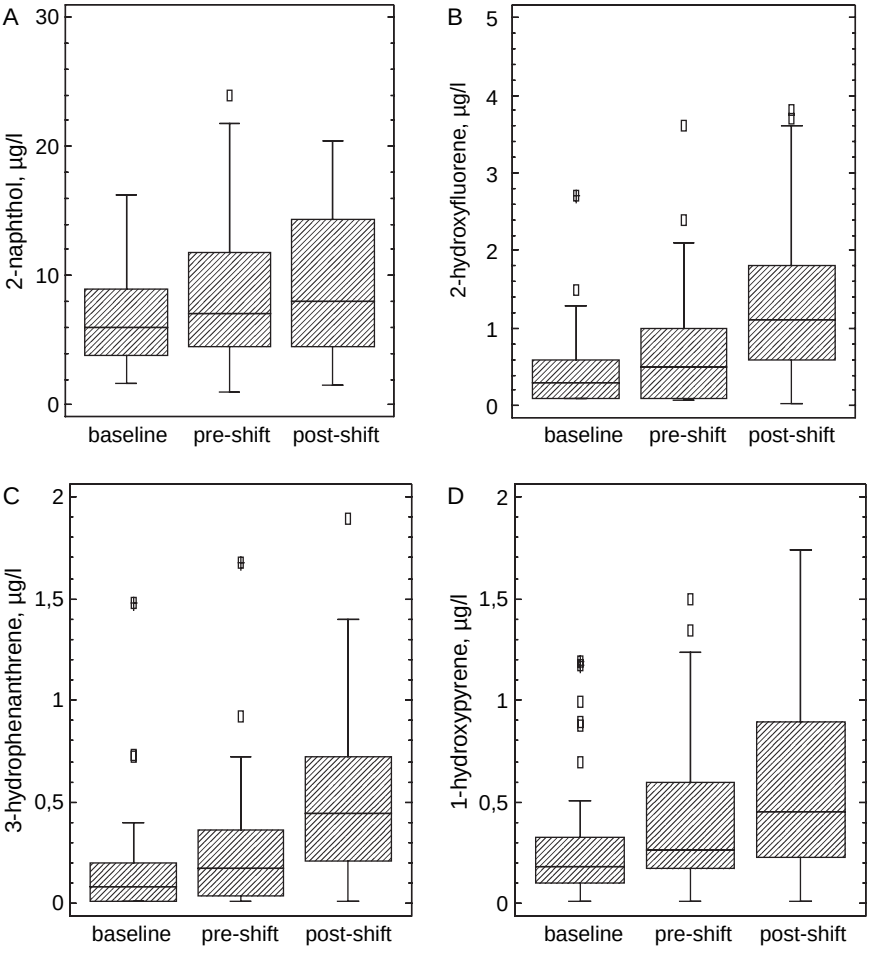


Figure 1. Box-and-whisker plots of distribution of excretion values of mono hydroxylated metabolites of PAHs observed in urine samples of non-smoking asphalt workers collected at three time points during the work week. (A) 2-naphthol, (B) 2-hydroxyfluorene, (C) 3-hydroxyphenanthrene, (D) 1-hydroxypyrene. Urinary concentrations are expressed in $\mu g\, l^{-1}$. The centre line within each box represents the median, the boxes represent the 25–75% percentile, the bars extend to the extremes, and the dots are statistical outliers (distance to box exceeds 1.5 times the box width).

*Relationships among urinary and environmental variables*

The relationships between different biomarkers and between biomarkers and air contaminants were dealt with separately in non-smokers and smokers. Pearson's correlation coefficients of significant associations between OH-PAHs within a single or in replicate samples, and between OH-PAHs and vapour-phase airborne contaminants are reported in Table III for non-smoking subjects. Within a single spot urine, statistically significant associations between metabolites were found (e.g. post-shift 3PHE vs. post-shift 1OHP, $r = 0.74$), except for 2NAP whose values correlated only incidentally with other OH-PAHs. Studying the within-subject variability of excretion in replicate samples, serial measurements of each of the individual metabolites were significantly correlated; the strongest associations were

Table III. Correlation matrix of urinary biomarkers and environmental exposure parameters for non-smokers ($n = 55$). Pearson correlation coefficients $r$ are shown for each comparison, based on log-transformed data. For abbreviations see Materials and Methods.

| | Biological markers | | | | | | | | | | | Indicators of environmental exposure | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | | | Pre-shift | | | | Post-shift | | | | | | |
| | 2FLE | 3PHE | 1OHP | 2NAP | 2FLE | 3PHE | 1OHP | 2NAP | 2FLE | 3PHE | 1OHP | V-PAC | FLE | PHE | PYR |
| *Baseline* | | | | | | | | | | | | | | | |
| 2NAP | ns | ns | ns | 0.54 | 0.26 | 0.29 | ns | 0.54 | ns | ns | ns | | | | |
| 2FLE | | 0.49 | 0.54 | ns | 0.55 | 0.44 | 0.42 | ns | 0.32 | ns | 0.42 | | | | |
| 3PHE | | | 0.67 | ns | 0.39 | 0.38 | 0.37 | ns | 0.27 | 0.35 | 0.36 | | | | |
| 1OHP | | | | ns | 0.40 | 0.41 | 0.53 | ns | 0.40 | 0.52 | 0.60 | | | | |
| *Pre-shift* | | | | | | | | | | | | | | | |
| 2NAP | | | | | 0.34 | 0.41 | 0.26 | 0.70 | 0.27 | ns | ns | ns | ns | ns | ns |
| 2FLE | | | | | | 0.60 | 0.62 | ns | 0.60 | 0.37 | 0.50 | 0.43 | 0.34 | 0.44 | 0.28 |
| 3PHE | | | | | | | 0.73 | ns | 0.47 | 0.48 | 0.58 | 0.59 | 0.50 | 0.55 | 0.35 |
| 1OHP | | | | | | | | 0.25 | 0.55 | 0.58 | 0.84 | 0.54 | 0.26 | 0.47 | 0.41 |
| *Post-shift* | | | | | | | | | | | | | | | |
| 2NAP | | | | | | | | | ns | ns | ns | ns | ns | ns | ns |
| 2FLE | | | | | | | | | | 0.49 | 0.60 | 0.47 | ns | 0.45 | 0.29 |
| 3PHE | | | | | | | | | | | 0.74 | 0.49 | 0.39 | 0.46 | 0.30 |
| 1OHP | | | | | | | | | | | | 0.69 | 0.35 | 0.57 | 0.49 |

ns, not significant.

found for 1OHP (i.e. pre- vs. post-shift, $r = 0.84$). Moreover, serial measurements of various OH-PAHs were significantly associated one with the other (i.e. pre-shift 3PHE vs. post-shift 1OHP, $r = 0.58$).

Air exposure to vapour-phase contaminants and biomarker levels correlated both in pre- and post-shift urine samples. Airborne NAP was an exception, as no associations were found between air NAP levels and urinary values of metabolites. Statistically significant correlations were found between 2FLE, 3PHE and 1-OHP and their parent compounds in air ($r = 0.28–0.57$) and V-PAC ($r = 0.43–0.69$). Linear models might be fitted to describe the relationships between log-transformed values of OH-PAHs and vapour-phase contaminants. As an example, the equation of the linear regression model between post-shift 1OHP ($y$) and V-PAC ($x$) is reported: $y = -2.66 + 1.04\ x$, $r = 0.69$, $p < 0.01$. The correlation coefficient $r$ indicates a fairly strong relationship between the variables; the $r$-squared statistic shows that about 50% of the variability in $y$ is explained by the fitted model. No significant association was found between particle-phase contaminants and urinary biomarkers levels.

In smokers, the statistical significance of associations between the excretion values of each OH-PAH with each other OH-PAH within a spot urine and in replicate samples was generally improved compared with non-smokers. In addition, in smoking CW, statistically significant positive associations were found between different metabolites and cotinine (2NAP $r = 0.63$; 2FLE $r = 0.58$, 3PHE $r = 0.62$, 1OHP $r = 0.57$). Conversely, the correlations with environmental parameters were severely worsened or missed (data not shown).

## Discussion

The assessment of exposure to asphalt emissions is of concern, because of their irritating and possibly carcinogenic properties (IPCS 2004). In the present study, we investigated the behaviour of some urinary OH-PAHs as biomarkers of occupational exposure of asphalt workers engaged in road construction. In AW, a marked trend towards a rise in OH-PAH excretion values during the work week was found. These progressively increasing excretion levels might be attributable to the cumulative uptake of PAHs as a consequence of repeated consecutive exposure.

It is worth noting that each metabolite exhibited a characteristic pattern of excretion in consecutive samples in line with the biological half-lives of the parent compounds, which lengthen in function of lipophilicity (NAP < FLE < PHE < PYR). In fact, the elimination potential of hydrophobic organic contaminants, such as PAHs, depends on their lipophilic properties, and chemicals with high lipophilicity tend to be excreted slowly from the human body (Dimitrov et al 2003). Limited increases in concentrations of 2NAP were observed both during the work shift and across the work week in AW. Such findings are not surprising, considering the low levels of airborne naphthalene experienced by our AW, which are not really different from that of urban environments (IPCS 1998) and only 15% higher than that of CW (respectively, 426 ng $m^{-3}$ vs. 376 ng $m^{-3}$). Otherwise, 2NAP values observed here are similar to the notable background excretion in the general population (Preuss et al. 2003). In short, 2NAP proved unsuccessful for the assessment of small differences between workers in a work environment like the present one. The concentrations of 2FLE and 3PHE rose progressively during the work week in AW; however, the increase of concentration occurring during the work shift was about 2-fold higher than that observed between

pre-shift and baseline samples. A biphasic excretion process involving two different half-lives might explain these findings: a short one (some hours) for the readily available FLE/PHE and a longer one (a few days) for the slowly available component (Jacob & Grimmer 1996, Hecht et al. 2005). These findings offer new information about the behaviour of 2FLE and 3PHE, whose kinetics in humans are not well known. To our knowledge, no other reports are found in the literature on FLE and PHE accumulation in the body during the work week at low exposure levels of airborne PAHs. The 2FLE concentrations are in acceptable agreement with those reported in the few papers on FLE metabolites (Smith et al. 2002, Toriba et al. 2003, Benowitz et al. 2005); this is the first report on 2FLE excretion in asphalt exposure. The 3PHE values found here are in the same range as those found by other authors both in environmental and occupational low-level exposures (Jacob et al. 1999, Kuusimäki et al. 2003, Väänänen et al. 2003, Carmella et al. 2004). In the case of 1OHP, a highly significant and gradual rise in concentration was also observed during the work week; the increases observed during the work shift and the work week were similar. The moderate increase found during the work shift might be at least in part attributable to the non-optimal timing of post-shift urine collection. It is known that the maximal excretion of 1OHP might be delayed by several hours after the end of daily exposure and thus urine collection immediately after the work shift does not allow proper determination of 1OHP levels arising from pyrene intake during the shift (Gendre at al. 2002, Lafontaine et al. 2002). The pre-shift increase observed consequent to consecutive days of exposure might be rationalised by taking into account the kinetics of 1OHP excretion. In humans, the excretion process of 1OHP is biphasic, showing two relatively long half-lives: 10–35 h for the readily available pyrene and up to 16 days for the slowly available component (Brzeznicki et al. 1997). Both elimination components indicate pyrene accumulation during the work week. The possible accumulation of parent PAHs into the body within the work week is also supported by the significantly higher values of 1OHP observed in non-smoking AW in comparison with CW in baseline urine samples collected on Monday morning, when over 60 h had elapsed since the end of occupational exposure. Our findings are in agreement with reports in occupational groups exposed to fairly different levels of PAHs (Bouchard & Viau 1999, Järvholm et al. 1999, Kato et al. 2004, McClean et al. 2004a, Väänänen et al. 2003, 2005, 2006). For instance, in highly exposed creosote workers, 1OHP concentrations measured at the end of the shift were lower than in the evening samples (6–9 h after work) and pre-shift concentrations were at times higher than at end-shift (Elovaara et al. 1995). In a group of low-level exposed pavers/roofers, a significant increase in the rate of 1OHP excretion over a 3-day period was observed (Hatjian et al. 1995, Toraason et al. 2001), and a similar increasing trend was verified also in hot-mix asphalt pavers engaged in highway construction (McClean et al. 2004b).

The excretion values of the studied urinary biomarkers in CW not exposed to asphalt were essentially the same as the values reported in the literature for non-exposed controls, both in non-smoking and smoking subjects (Gündel et al. 1996, Hollender et al. 2000, Heudorf & Angerer 2001, Preuss et al. 2003, 2005, Carmella et al. 2004, Benowitz et al. 2005). A modest trend of increasing excretion during the course of the work week was also observed in these workers. This might be because of exposure to diesel exhaust emitted from mechanical earth-movers (Adonis et al. 2003,

Kuusimäki et al. 2003). The small number of subjects evaluated may possibly account for the inability to detect significant differences consistently.

Substantial inter-individual variability in urinary OH-PAH levels was observed in our study population. This variability can be accounted for by considering that the urinary concentration of each metabolite is the result of complex interactions among many factors. Differential contribution to the total dose of PAHs by various exposure routes (inhalatory, gastric and dermal), chemico-physical and toxicological properties of the parent compound, genotypic and phenotypic variability, hygienic practices, occupational and life environment, altogether concur to modulate the individual spectrum and abundance of metabolites (Bieniek 1994, Carmella et al. 1995, Gerde et al. 1998, Lee et al. 2001, Elovaara et al. 2003, Rihs et al. 2005). To avoid peaks of excretion due to excessive dietary intake, study subjects abstained from PAH-rich food but, of course, we could not eliminate the background exposure that all people experience. A further variability factor could be that, owing to practical reasons, the collection of pre- and post-shift urine was not invariably done for all subjects on the same day of the work week after a constant number of consecutive days at work, but by chance, after 2 or 3 days or, in a few instances, even 4 days. As a consequence of a possible bioaccumulation of parent PAHs from the preceding shift that were not fully bio-transformed, the excretion values of the metabolites both in pre- and post-shift samples might well change in connection with the number of elapsed consecutive days of exposure to bitumen emissions. Recently, in asphalt exposure, special emphasis was given to skin contamination, as many reports suggest that skin deposition of PAHs might significantly contribute to the total intake (McClean et al. 2004b, Väänänen et al. 2005). At the present time, we cannot give an estimate of the relevance of dermal deposition in the studied workers, because statistical processing of skin contamination data from our study is currently in progress.

Tobacco smoking is known to concur substantially with PAH uptake in general (IARC 1987). There is general agreement on its influence on the excretion levels of 2NAP and 1OHP, which in smokers are consistently higher (2–3-fold) than in non-smokers; however, conflicting results are reported in respect to phenanthrene metabolites (Martin et al. 1989, Jacob et al. 1999, Kuusimäki et al. 2003). In the present study, the smokers invariably exhibited higher OH-PAH excretion values than their non-smoking colleagues. Smoking increased, to a lesser extent, the urinary levels of 2NAP (2-fold) and 1OHP (3-fold) compared to 2FLE- and 3PHE-induced elevations (10–15-fold). In addition, the positive associations among cotinine and OH-PAHs indicated a significant dose-response relationship and reflected the increasing intake of PAHs in smokers according to the intensity of their smoking habit.

To obtain a rough evaluation of the relative contribution to PAH intake from cigarette smoking or from exposure to asphalt emissions, we compared OH-PAH excretion in smoking CW with that in non-smoking AW during the work shift. Median concentrations of OH-PAHs of smoking CW were always higher than those found in non-smoking AW, suggesting that the amount of PAHs inhaled from cigarettes apparently exceeded that of occupational exposure. Otherwise, about 5% of non-smoking AW had higher excretion values of pre- and post-shift 1OHP than the highest individual value observed in smoking CW. According to our data, a meaningful interpretation of biological monitoring of occupational exposure to PAHs in asphalt emissions is therefore only feasible if smoking habits are carefully taken into account.

The significant correlations between airborne contamination and metabolite concentrations in the urine of non-smokers indicate a reasonable coherency on an individual basis for the excretion of metabolites as a consequence of environmental exposure. The positive parameters confirm that OH-PAH excretion increases with an increasing burden of inhaled PAHs. Currently, urinary 1OHP is a widely used sentinel biological indicator of exposure to PAHs, generally regarded as the standard *par excellence* for the evaluation of short-term occupational exposure (Jongeneelen 2001). In our study, it turned out to be a relatively sensitive biomarker for the assessment of low-level exposure because an approximate 30-fold difference in airborne PYR levels between AW and CW gave rise to only a 4-fold increase in 1OHP concentration. Both 2FLE and 3PHE proved to be more responsive biomarkers; in fact, a 5-fold increase in airborne FLE or PHE levels of AW caused a 3–4-fold parallel increase in metabolite excretion values compared to CW. These results support the observation that phenanthrene metabolites proved to be better biological monitoring variables than 1OHP in PAH-exposed workers (Popp et al. 1997, Rihs et al. 2005) and offer the interesting premises that both 2FLE and 3PHE might serve as useful biomarkers at low levels of vapour-phase PAH exposure.

The within-subject mutual associations between different OH-PAHs both in individual and serial urine samples might be explained by a common origin of parent PAHs. In fact, when considering the primary PAH individual source of intake (food, occupational exposure, smoking status), different relationships between biomarkers were found. In non-smoking CW, in whom food is supposedly the major PAH contributor, a sole association was found between 3PHE and 1OHP. In non-smoking AW, in whom exposure to asphalt emission should constitute an important additional PAH source, 2FLE, 3PHE and 1OHP, but not 2NAP, were significantly associated with each other. In smokers, whose smoking habit greatly contributed to total PAH intake, highly significant associations between all four metabolites were found. Accordingly, the study of relationships and relative abundances of multiple urinary metabolites might help towards improved understanding of the apportioning of different sources to individual PAH body dose.

As regards metabolites of particle-phase PAHs (6CHR and 3BAP), their levels were always below the analytical limit of detection. This is in good accordance with the very low levels of high-boiling PAHs in air (median airborne levels in the range 0.1–1.2 ng $m^{-3}$). On the other hand, it is also possible that the use of more sensitive analytical methods might allow the quantification of these compounds at these very low levels of exposure (Simon et al. 2000).In conclusion, we demonstrated the applicability of OH-PAHs for monitoring exposure to asphalt emissions. Our results show that the work-related uptake of PAC and PAHs was low in AW, although significantly greater than in CW, and urinary metabolite excretions were fairly well correlated with environmental parameters. Finally, our study, in highlighting the importance of selecting appropriate timing for specimen collection for biological monitoring, might serve as a basis for future research on this theme.

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