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ORIGINAL ARTICLE

Occupational exposure to polycyclic aromatic hydrocarbons: relations between atmospheric mixtures, urinary metabolites and sampling times

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Abstract
Purpose Occupational exposure to polycyclic aromatic hydrocarbons (PAHs) can be assessed by either air monitoring or biomonitoring using urinary 1-hydroxypyrene (1-OHP) or 3-hydroxybenzo(a)pyrene (3-OHBaP). The aim of this study was to understand the links between atmospheric PAHs and urinary metabolites, in order to improve the biomonitoring strategy for assessing carcinogenic risk.
Methods Personal air sampling for pyrene and BaP measurements, and urines for 1-OHP and 3-OHBaP analyses of seven workers from electrode production plant were collected every day of the working week.
Results High variability of atmospheric levels between activities and between days was observed, especially for gaseous pyrene. No correlation was found between urinary metabolites: 1-OHP maximum levels occurred for "electrode extrusion" activity; those of 3-OHBaP occurred for "raw materials dispatcher." Sixty percentage of 3-OHBaP maximum levels were observed in urines collected at the beginning of shift the last workday. Those of 1-OHP occurred at different sampling times, depending on the gaseous pyrene levels (not stopped by P3 respirators). Dermal absorption of PAHs was confirmed by significant effect of particulate pyrene on 1-OHP in the samples collected the morning of the following day ($p < 0.02$, $n = 25$).
Conclusions Lack of correlation between metabolites concentrations emphasizes the non-relevance of 1-OHP, from a non-carcinogenic gaseous and particulate compound, and the great interest of 3-OHBaP, from carcinogenic BaP. Its slower urinary elimination prevents the risk of exposure underestimation, and urinary analysis should be performed at the beginning of shift the end of working week, especially in case of high exposure variability.

Keywords Polycyclic aromatic hydrocarbons · Occupational exposure · Biomonitoring · 1-hydroxypyrene (CAS: 5315-79-7) · 3-hydroxybenzo(a)pyrene (CAS: 13345-21-6)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants emitted during incomplete combustion of organic matter and coal or petroleum distillation. They are found in the atmosphere as complex gaseous and particulate mixtures, whose composition varies considerably according to the emission source. Within this chemical family, benzo(a)pyrene (BaP) is classified as carcinogenic to humans (group 1) by the International Agency for Research on Cancer (IARC 2010). Humans are exposed through tobacco consumption, diet, urban pollution and industrial emissions. Occupational exposures occurring during coal-tar distillation, paving and roofing with coal-tar pitch or aluminum production are responsible for lung, skin and bladder cancers (IARC 2012).

To assess occupational health risk, the monitoring of PAHs exposure includes the measurement of airborne

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concentrations and biological monitoring using biomarkers. In the industrial sector of carbon electrode production, BaP median concentrations range from 0.4 µg/m^3 for maintenance activities to 1.1 µg/m^3 for cathode impregnation with liquid pitch, and in coke plants levels up to 6.2 µg/m^3 (Angerer et al. 1997; Van Delft et al. 1998; Van Schooten et al. 1995). These studies showed the great variability of atmospheric levels of PAHs between and within industries. However, air monitoring only assesses potential inhalation exposure and does not take into account skin absorption and the wearing of personal protection equipment (Van Rooij et al. 1993).

Quantification of the internal dose is indeed the best way to assess occupational exposure and health risks. Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, has been proposed by Jongeneelen for the biomonitoring of PAHs exposure (Jongeneelen et al. 1985). The analysis is simple and reliable, urinary levels are frequently measured in the range of µmole/mole of creatinine (µmol/mol), but it assesses pyrene absorption, a non-carcinogenic PAH. Urinary 3-hydroxybenzo(a)pyrene (3-OHBaP), a metabolite of BaP, would be a more appropriate biomarker to assess carcinogenic PAHs exposure, because urinary concentrations are correlated with DNA adducts in the lungs of rats after intravenous injection of BaP (Marie-Desvergne et al. 2010). However, to perform biomonitoring of occupational carcinogenic PAHs exposure using this metabolite, a highly sensitive routine analytical method is necessary, because urine levels are 1000 times lower than those of 1-OHP (Barbeau et al. 2011). Animal studies have shown that urinary concentrations of 3-OHBaP represent only 0.1–0.2 % of the BaP dose, due to a complex metabolism producing several different metabolites that are mainly excreted in feces (Marie et al. 2010; Payan et al. 2009). Maximum urinary levels found in workers were 5 nmol/mol and 19.5 µmol/mol for 3-OHBaP and 1-OHP, respectively, in carbon anode production; 5.3 nmol/mol and 7 µmol/mol in carbon cathode production; and 1.1 nmol/mol and 2.9 µmol/mol in silicon production (Barbeau et al. 2014). Mean end-of-shift levels of urinary 3-OHBaP were as low as 0.2 nmol/mol in coke production workers and 0.5 nmol/mol for workers on a graphite electrode production line (Forster et al. 2008).

Knowledge of the elimination kinetics of the urinary metabolite, used as an exposure biomarker, is essential in order to define the urine sampling strategy. Levels of urinary 1-OHP reach maximum values 3–9 h after the end of work (Bouchard and Viau 1999), while the excretion peak of 3-OHBaP occurs on average 15 h following occupational exposure, with a range of 3–24 h post-shift, depending on the tasks considered (Gendre et al. 2002, 2004). The delay in urinary elimination of 3-OHBaP is partly explained by the storage of BaP in organism and the retention of its metabolite in kidneys (Marie et al. 2010). However, absorption routes and exposure frequency also influence biomarker elimination. The mean urinary half-life of 1-OHP reached 3.9 h in the case of ingestion of barbecued chicken, compared with 6 h after cigarette smoke (Li et al. 2012; St Helen et al. 2012). This value increased to 13 h after occupational exposure if the pyrene entered the organism via the skin, such as during creosote or asphalt application (Sobus et al. 2009b; Viau et al. 1995; Viau and Vyskocil 1995). A pre-shift sample on Monday and a pre-shift or post-shift sample on Friday should be collected to analyze 1-OHP when the main route of exposure is via the skin or pulmonary absorption, respectively (Bouchard and Viau 1999). However, it is difficult to discriminate between the major absorption routes, because pyrene is emitted into the atmosphere in gaseous form which is mainly absorbed through the lungs and in particulate form which can be absorbed both via a pulmonary route and a dermal route after deposition of particles on the skin (Sobus et al. 2009a). Although the elimination kinetics of 3-OHBaP has less been studied than the one of 1-OHP, Gendre et al. (2004) proposed to collect urinary samples at the beginning of shift the day after occupational exposure, to take into account its excretion delay. However, no difference was observed between the 3-OHBaP levels found at the end of the penultimate workday shift and those at the beginning of the last workday shift, among more than 100 workers exposed consecutively throughout the working week (Barbeau et al. 2014). In this last study, BaP exposure occurred over several consecutive days, while, in the study of Gendre et al. (2004), workers were exposed for a single day.

The aim of the present work was to better understand the factors affecting the variability of urinary levels of 3-OHBaP and 1-OHP among workers during a whole working week, in order to select the best biomarker and to set the best sampling time for assessing carcinogenic risk. Pre-shift and post-shift urine samples were collected every day of the working week as well as individual air samples. Urinary levels of the two metabolites measured at different sampling times were compared to determine the relative importance of cutaneous and respiratory absorption routes. Urinary concentrations were also compared with daily airborne concentrations of BaP and pyrene (both gaseous and particulate pyrene) in order to study the influence of the chemical composition of the aerosol, and in particular the gaseous to particulate PAHs ratio.

Materials and methods

Study population

The subjects were seven non-smoking, healthy, male volunteers, between 30- and 60-year old, working in a pre-baked

Table 1 Activities and protective equipment for each worker, each day (D) of the working week

Day of the working week	Activity	Collective protection	Respiratory protection	Skin protection
Carbon sector				
Worker 1 (W1)				
D1	Installation cleaning Carbon electrode extrusion	Air curtain	P3 non-powered	None
D2–D3	Carbon electrode extrusion		None	
D4	Installation cleaning Carbon electrode extrusion			
Worker 2 (W2)				
D1	Installation cleaning	None	None	Handling gloves
D2			P3 powered	
D3	Overhead crane operator		None	
D4			P3 powered	
Worker 3 (W3)				
D1–D4	Mobile raw material dispatcher	None	P3 non-powered	Handling gloves
Worker 4 (W4)				
D1–D5	Non-mobile raw material dispatcher	None	P3 non-powered	Handling gloves
Graphite sector				
Worker 5 (W5)				
D1	Mobile raw material dispatcher	None	None	Handling gloves
D2–D5			P3 non-powered (paper)	
Worker 6 (W6)				
D1–D4	Safety rounds in the installations	None	P3 powered	None
D5	Administrative tasks			
Worker 7 (W7)				
D1	Forklift driver	None	P3 non-powered	Handling gloves
D2	Graphite electrode extrusion			
D3	Outdoor painting		None	
D4	Graphite electrode extrusion		P3 non-powered	
D5	Installation cleaning Graphite electrode extrusion			

electrode production plant. Workers 1–4 and 5–7 were involved in carbon and graphite sectors, respectively. A questionnaire was completed by each worker in order to inform them about the study and obtain their consent and to collect detailed data about products, processes and daily tasks performed, as well as the collective and personal protective equipment (Table 1).

While activities of workers 3–5 were relatively unchanged, those of workers 1, 2, 6 and 7 varied during the week. Installation cleaning consists of sweeping and air blowing deposited particulate matter. Electrode extrusion refers to carbon or graphite electrode molding with a hot press. The overhead crane operator and the forklift driver move and store the electrodes. Dispatchers supply the press with raw materials from different places (mobile dispatcher) or from one workstation (non-mobile dispatcher). Safety rounds of the installations correspond to checking the proper functioning of technical facilities, while administrative tasks are performed in an office.

There was no collective protective equipment in the workplaces except for worker 1 (W1) who was in a cabin, where PAHs emissions were confined by an air curtain during carbon electrode extrusion. Respirators were consistently of type P3. W5 wore a disposable paper mask, while the other workers wore reusable respirators, either non-powered rubber half masks (W1, W3, W4, W7) or hoods powered with cartridges worn at the belt (W2 and W6). Handling gloves were worn by all workers, except for W1 and W6.

Urine and atmospheric sample collection

All samples were collected under the responsibility of the occupational health physician of the company as part of the routine follow-up of workers. Urine samples were collected

at the beginning (BS) and at the end of shift (ES) every day of the working week. Three subjects (W1, W2 and W3) worked 4 days and provided eight samples while four subjects (W4, W5, W6 and W7) worked 5 days and provided ten samples. Samples were collected in 50-mL propylene bottles and stored at −20 °C until analysis.

For each worker, personal samples were collected every workday, over 7 h, according to the methods recommended in France (AFNOR 1995). An opaque polypropylene cassette holding a 37-mm-diameter quartz filter from Whatman (Clifton, USA) was connected in series to an ORBO 43 Supelpak-20 XAD2 tube from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Air sample squeezed through filter, where BaP and pyrene present in the particulate phase (pPyr) were trapped, before to reach the XAD2 cartridge where gaseous pyrene (gPyr) was adsorbed. Filters are designed to retain particles with a diameter less than 1 µm. This sampling device was connected to a pump whose flow rate was regulated at 1 l/min. The stability of the air flow during sampling was checked by comparing rates at the beginning and the end of sampling and a variation greater than 5 % resulted in the exclusion of the sample. Afterward, the sample material was stored at −20 °C until analysis.

Urinary analyses

After thawing the urine overnight, the 3-OHBaP analysis was conducted using the method previously described by Barbeau et al. (2011). Briefly, a 10 mL aliquot of urine was diluted in acetate buffer before enzymatic hydrolysis by β-glucuronidase/arylsulfatase from Roche (Mannheim, Germany). The sample was purified and concentrated by automated solid phase extraction from Gilson (Villiers-le-Bel, France) using Sep-Pak C18, 500 mg with a capacity of 6 mL from Waters (Guyancourt, France). Urinary 3-OHBaP was quantified by high-pressure liquid chromatography coupled with fluorescence detection (HPLC-FLD) from Waters. The "heart-cut" technique was used, with a first sample separation performed on a Supelcosil LC-18 purification column (50 mm × 4 mm, 5 µm) from Sigma-Aldrich and a second separation performed on a PAH-C18 analytical column (250 mm × 3 mm, 5 µm) from Waters. Excitation and emission wavelengths were fixed at 425 and 465 nm, respectively. The limit of quantification (LQ) was 0.2 pmol/L (0.05 ng/L).

Urinary 1-OHP was determined using a modified method of that previously described by Jongeneelen et al. (1988). Briefly, a 2 mL aliquot of urine was diluted in acetate buffer before enzymatic hydrolysis and directly injected into a HPLC-FLD system from Waters. Online extraction of 1-OHP was performed with a Supelguard C18 column from Sigma-Aldrich and elution was performed with a LiChrospher C18 column from Merck (Billerica, USA). Excitation and emission wavelengths were fixed at 333 and 390 nm, respectively. The LQ was 91.6 pmol/L (20 ng/L). The laboratory meets the German Society for Occupational and Environmental Medicine external quality control system standards for 1-OHP.

Urinary creatinine was determined by Jaffe's method in a 2 mL aliquot of urine. All metabolites' concentrations were adjusted to urinary creatinine (mol/mol).

Atmospheric sample analysis

Fifteen milliliters of HPLC grade dichloromethane was added to each filter. After ultrasonic extraction for 30 min, the filter was removed, and the solvent was evaporated using a rotary evaporator at 30 °C. The dry residue was taken up in 1 mL of HPLC grade acetonitrile and filtered through a 0.22-µm filter before analysis. The XAD2 tube was eluted with 2 mL acetonitrile. PAHs were measured by HPLC-FLD using a HPLC Alliance 2695 separation module and a 2475 Multi λ Fluorescence Detector from Waters. Chromatographic separation was performed by injecting 30 µL on a PAH-C18 column (250 mm × 3 mm, 5 µm) from Waters and elution with an acetonitrile/water gradient. The column oven temperature was set at 32 °C. The wavelengths (excitation–emission) used were 333–390 and 296–405 nm for Pyr and BaP, respectively. The LQ was 0.25 pmol/tube (0.05 ng/tube) for gPyr, 0.25 and 0.20 pmol/filter (0.05 ng/filter) for pPyr and BaP, respectively.

Data analyses

Concentrations of metabolites and atmospheric PAHs lower than the LQ were assigned a value equal to half the LQ for graphics and statistical analyses. Total pyrene (tPyr) was the sum of gPyr and pPyr levels. Ratios between urinary concentrations of 3-OHBaP at the end of shift +16 h (ES16) and 1-OHP at ES (ES16/ES), or between urinary concentrations of the two metabolites obtained at the same sampling time, at ES (ES/ES) or ES16 (ES16/ES16), were calculated. Nonparametric Wilcoxon test and Spearman coefficient (rhô) were performed with SPSS Statistics 22 software (IBM) to compare paired samples and to study correlations, respectively. Due to the repeated measures sampling design, linear mixed effects models were performed with R 3.1.2 software (R Foundation for Statistical Computing) to estimate relations between urinary metabolites concentrations and atmospheric PAHs levels, using the "lme" function from "nlme" package (Pinheiro and Bates 2000). Log-transformed values of atmospheric PAHs levels and urinary metabolites concentrations at different sampling times were included as fixed effects. Workdays,

i.e., the repetitions, were included as random effect. For all tests, p values below 0.05 were considered significant.

Results

Atmospheric PAHs measurements

All BaP analyses from elution of XAD2 tubes were below the LQ, showing that BaP was trapped on filters.

Individual levels of gPyr, pPyr and BaP and concentrations ratios of gPyr/pPyr, BaP/gPyr, BaP/pPyr and BaP/tPyr are shown in Table 2.

Levels of gPyr ranged from 10.3 to 876.3 ng/m^3 in the carbon sector and from 0.9 to 143.4 ng/m^3 in the graphite sector, over the 5 days. The highest levels were found in carbon electrode extrusion (W1) and the lowest levels during raw material dispatching in the graphite sector (W5). pPyr levels were less variable than those of gPyr and ranged from 27.1 to 289.8 ng/m^3 in the carbon sector and from 7.2 to 239.3 ng/m^3 in the graphite sector. The highest level was found in carbon sector raw material dispatching (W4) and the lowest during safety rounds in the graphite sector (W6). Pyrene levels also varied between days of the working week for a given worker. The highest variability of gPyr was found for W7, with a relative standard deviation (RSD) close to 140 %, while that of pPyr was found for W2, with a RSD_{max} equal to 95 %. pPyr levels were higher than those of gPyr ($p < 0.05$), with mean gPyr/pPyr ratios lower than 0.5, except for W4 and W1. Indeed, carbon electrode extrusion (W1) was responsible for the emission of significant amounts of gaseous PAHs, with an average gPyr/pPyr ratio close to 3.8. This ratio was highly variable between days of the working week, especially for W3 in the carbon sector (RSD > 110 %) and for W6 and W7 in the graphite sector (RSD ≈ 140 %).

The variability of BaP airborne concentrations was similar to that of pPyr and ranged from 26.9 to 425 ng/m^3 in the carbon sector and from 8.8 to 231.7 ng/m^3 in the graphite sector. For the two sectors, the highest levels were found for dispatching tasks. Levels of BaP were less variable than those of gPyr and pPyr between days of the working week, with a RSD_{max} lower than 80 % for W7. While BaP/gPyr ratios were very variable from one task to another (from 0.23 to 49.03) and between days (with RSD greater than 80 %, except for W3), BaP/pPyr ratios were much more homogenous between different tasks and days (RSD ranged from 14 to 54 % depending on the worker). Finally, average BaP/tPyr ratios were close to one, revealing an amount of BaP similar to that of tPyr ($p > 0.05$), except for carbon electrode extrusion (W1) where BaP was six times lower than tPyr.

Correlations were nonsignificant between atmospheric concentrations of gPyr and pPyr (rhô = 0.24, $p > 0.05$, $n = 32$) and between gPyr and BaP (rhô = 0.18, $p > 0.05$, $n = 32$), while pPyr and BaP concentrations were well correlated (rhô = 0.78, $p < 0.05$, $n = 32$). Thus, correlation between tPyr and BaP was significant but poor (rhô = 0.48, $p < 0.05$, $n = 32$).

Urinary concentrations of 3-OHBaP and 1-OHP

Only three out of 64 samples were below the LQ for 3-OHBaP and none for 1-OHP. Levels were higher in urine collected at the end of shift the end of week (ESEW) than at the beginning of shift the beginning of week (BSBW) for 1-OHP ($p < 0.02$) and 3-OHBaP ($p < 0.02$; Table 3).

3-OHBaP levels ranged from 0.02 to 0.80 nmol/mol. W1 had very low levels throughout the week, while the highest level was measured for W6 at the end of the working week. Maximum 3-OHBaP levels were observed at the beginning of the last workday shift (BSEW) for four out of seven workers (W2, W3, W4 and W6) and on ES for the other workers (W1, W5 and W7). No significant difference was found between the urinary concentrations in samples obtained at BSEW and those from ES on the day before ($p > 0.05$, $n = 7$).

Urinary concentrations of 1-OHP were more variable and ranged between 0.08 and 2.08 µmol/mol. The lowest levels were found for W2 and W4. W1 had a very high level (higher than 2 µmol/mol) at the end of the second workday. The maximum always occurred at ES, for four workers on the second day (W1, W3, W4 and W7), for two at the end of the week (W5 and W6), for one on the third (W2) and another on the fourth day (W4). The concentrations of 1-OHP were significantly lower in urine samples taken at BSEW than in those from ES the day before ($p < 0.02$, $n = 7$).

Median values of 3-OHBaP/1-OHP ratios were close for ES16/ES, ES/ES and ES16/ES16, for a given worker. W1 showed the lowest values, from 0.03 to 0.04, while those of the others ranged from 0.23 (W2) to 0.47 (W3). Ratios were also variable between days of the working week: RSD ranged from 14 % (W2) to 66 % (W6) for ES16/ES, from 31 % (W5) to 81 % (W6) for ES/ES and from 20 % (W3) to 62 % (W6) for ES16/ES16.

Urinary concentrations of the two metabolites and corresponding atmospheric pyrene and BaP levels during the working week are summarized in Fig. 1. There was no regular increase in urinary concentrations throughout the working week, and levels found at the ES the second day were close to those at ESEW. Only W5 for 1-OHP and W6 for the two metabolites showed highest biomarkers levels at the end of the working week. Conversely, the 1-OHP level of W1 was three times higher at ES-D2 than at ESEW. After the first significant day of exposure, the urinary concentrations

Table 2 Airborne PAHs data for each day of the working week

	Daily levels (ng/m^3)			Concentration ratios (median [min–max])			
	gPyr	pPyr	BaP	gPyr/pPyr	BaP/gPyr	BaP/pPyr	BaP/tPyr
Carbon sector							
W1							
D1	623.6	227.6	92.4	**3.51** [1.32–6.84]	**0.16** [0.10–0.51]	**0.55** [0.41–1.19]	**0.13** [0.08–0.29]
D2	876.3	205.0	85.7				
D3	314.8	46.0	54.7				
D4	143.7	109.0	73.6				
W2							
D1	41.1	271.5	218.3	**0.30** [0.09–0.54]	**4.30** [2.20–17.33]	**1.31** [0.80–1.78]	**0.93** [0.68–1.49]
D2	12.2	27.1	26.9				
D3	30.8	56.9	101.2				
D4	10.3	109.7	178.5				
W3							
D1	34.2	139.0	248.6	**0.29** [0.25–0.47]	**6.16** [2.82–7.28]	**1.72** [1.33–1.91]	**1.37** [0.90–1.44]
D2	39.9	122.9	234.4				
D3	52.7	206.3	339.0				
D4	46.3	97.9	130.4				
W4							
D1	64.5	289.8	425.0	**0.51** [0.22–3.59]	**3.50** [0.51–6.59]	**1.77** [1.32–1.84]	**1.18** [0.40–1.21]
D2	114.6	32.0	58.8				
D3	74.3	147.0	260.2				
D4	66.2	133.8	241.1				
D5	122.4	83.4	110.1				
Graphite sector							
W5							
D1	7.7	67.5	70.2	**0.08** [0.01–0.16]	**17.52** [7.32–139.30]	**1.04** [0.56–1.47]	**0.96** [0.56–1.36]
D2	0.9	120.1	67.8				
D3	9.2	109.9	161.5				
D4	1.7	239.3	231.7				
D5	12.5	76.2	91.4				
W6							
D1	10.9	50.3	72.7	**0.22** [0.05–1.52]	**6.66** [0.80–22.65]	**1.22** [0.69–1.52]	**1.00** [0.48–1.24]
D2	11.0	7.2	8.8				
D3	4.9	106.4	110.9				
D4	22.3	110.9	76.9				
D5	8.9	39.7	60.3				
W7							
D1	9.4	50.1	55.9	**0.17** [0.10–1.41]	**5.92** [0.44–12.84]	**0.91** [0.50–1.33]	**0.79** [0.26–1.20]
D2	143.4	101.9	63.4				
D3	7.9	54.8	49.8				
D4	29.5	173.7	86.6				
D5	17.9	173.1	230.0				

Bold values indicate medians

W worker, *D* day

of both biomarkers did not come back to their basal levels the following morning. This was observed for both metabolites after the first workday for W1 to W6 and after the second workday for W7, and for 3-OHBaP, after the penultimate day for W3 and W4 and also after the penultimate day of W6 although he had been previously exposed.

Table 3 Urinary concentrations and ratios of 3-OHBaP and 1-OHP in pre-shift and post-shift urine samples during the working week

	BSBW–ESEW; **max** (sampling time)		3-OHBaP/1-OHP ratios (**median** [min–max])		
	3-OHBaP (nmol/mol)	1-OHP (μmol/mol)	ES16/ES	ES/ES	ES16/ES16
W1	0.02–0.05 **0.05** *(ES-D2/D3/EW)*	0.11–0.70 **2.08** *(ES-D2)*	**0.04** [0.02–0.07]	**0.03** [0.02–0.07]	**0.04** [0.02–0.07]
W2	0.04–0.14 **0.15** *(BSEW)*	0.08–0.35 **0.46** *(ES-D3)*	**0.30** [0.25–0.33]	**0.23** [0.07–0.40]	**0.23** [0.22–0.40]
W3	0.06–0.23 **0.40** *(BSEW)*	0.11–0.58 **0.72** *(ES-D2)*	**0.47** [0.28–0.54]	**0.34** [0.03–0.41]	**0.40** [0.28–0.41]
W4	0.08–0.12 **0.19** *(BSEW)*	0.12–0.40 **0.43** *(ES-D2/D4)*	**0.26** [0.23–0.46]	**0.26** [0.14–0.46]	**0.24** [0.14–0.30]
W5	0.09–0.16 **0.24** *(ES-D2)*	0.20–0.73 **0.73** *(ESEW)*	**0.45** [0.25–0.89]	**0.45** [0.22–0.51]	**0.39** [0.22–0.51]
W6	<LQ–0.64 **0.80** *(BSEW)*	0.1–0.86 **0.86** *(ESEW)*	**0.44** [0.20–0.86]	**0.30** [0.04–0.74]	**0.31** [0.20–0.74]
W7	0.07–0.29 **0.29** *(ESEW)*	0.22–0.69 **0.83** *(ES-D2)*	**0.41** [0.31–0.58]	**0.34** [0.18–0.54]	**0.35** [0.18–0.54]

Bold values indicate maximum urinary concentrations

D day, *BSBW* beginning of shift, beginning of week, *BSEW* beginning of shift, end of week, *ESEW* end of shift, end of week, *BS* beginning of shift, *ES* end of shift, *ES16* end of shift + 16 h

Relations between atmospheric levels and urinary concentrations

Atmospheric levels of gPyr, pPyr and BaP during the first workday were not correlated with urinary concentrations of 1-OHP and 3-OHBaP in samples collected at ES-D1 and BS-D2. Considering all measurements throughout the working week, significant positive effects of 1-OHP in BS urines and atmospheric gPyr levels were observed on 1-OHP concentrations in ES samples. However, 1-OHP concentrations on ES16 were linked only with 1-OHP concentrations on ES and pPyr levels (Table 4). On the other hand, no significant effect of atmospheric BaP was observed on urinary concentration of 3-OHBaP, regardless of the sampling time, but 3-OHBaP levels on ES were linked with those on BS (Table 5).

Discussion

Atmospheric levels of PAHs are higher in the carbon sector than in the graphite sector, with nearly 50 % of BaP values exceeding 150 ng/m^3 which is the occupational exposure limit (OEL) recommended by the French health insurance agency. This is probably due to the disrepair of facilities in the carbon sector compared with the graphite sector and the use of different raw materials. However, these levels are lower than those previously reported in the same industrial sector (Petry et al. 1996), due to improvements in industrial processes and changes in the raw materials used in the last 20 years.

The high variability of PAHs is due to occupational activities that were very different from one individual to another and also from one day to another for the same worker. While the levels of the particulate phase (pPyr and BaP) correlate well, their variations are very different and lower than those of the gas phase (the RSD of gPyr is three times higher than that of pPyr and BaP). This difference has been already reported for workers exposed to creosote during one working week (Elovaara et al. 1995) and is due to the different sources of emission. Indeed, the maximum levels of particulate PAHs had been measured at the raw material dispatchers, while those of gPyr were measured during the "electrode extrusion" process in both sectors (every day for W1 and D2 for W7). At the dispatchers, the handling of raw carbon material containing coal-tar pitch generates a large amount of particulate PAHs, whereas during extrusion, the heat requested for electrode molding leads to the release of high amounts of gaseous PAHs. Similarly, levels of BaP and pPyr vary by one order of magnitude between two consecutive days of facility cleaning and safety checks (D1 and D2 for W2 and W6, respectively), while they are very close for gPyr. These results explain the large variability in gPyr/pPyr ratios depending on the workers activity and the day of the week, which had already been reported in this sector (Petry et al. 1996) or in an artificial shooting target factory (Lafontaine et al. 2000). Bentsen et al. (1998) study, conducted during the production of electrodes, is the only one to show a good correlation between the levels of BaP and tPyr because the proportion of pPyr in the mixture was greater. These results illustrate the various sources of variability of the chemical composition of atmospheric aerosols and highlight the difficulty of finding a pollutant tracer other than BaP to estimate the carcinogenic risk resulting from these mixtures.

In order to assess the real occupational exposures, biological monitoring that takes into account dermal absorption is essential. Urinary 3-OHBaP is an accurate biomarker to evaluate carcinogenic exposure. Recently, a reliable and specific analytical method was developed

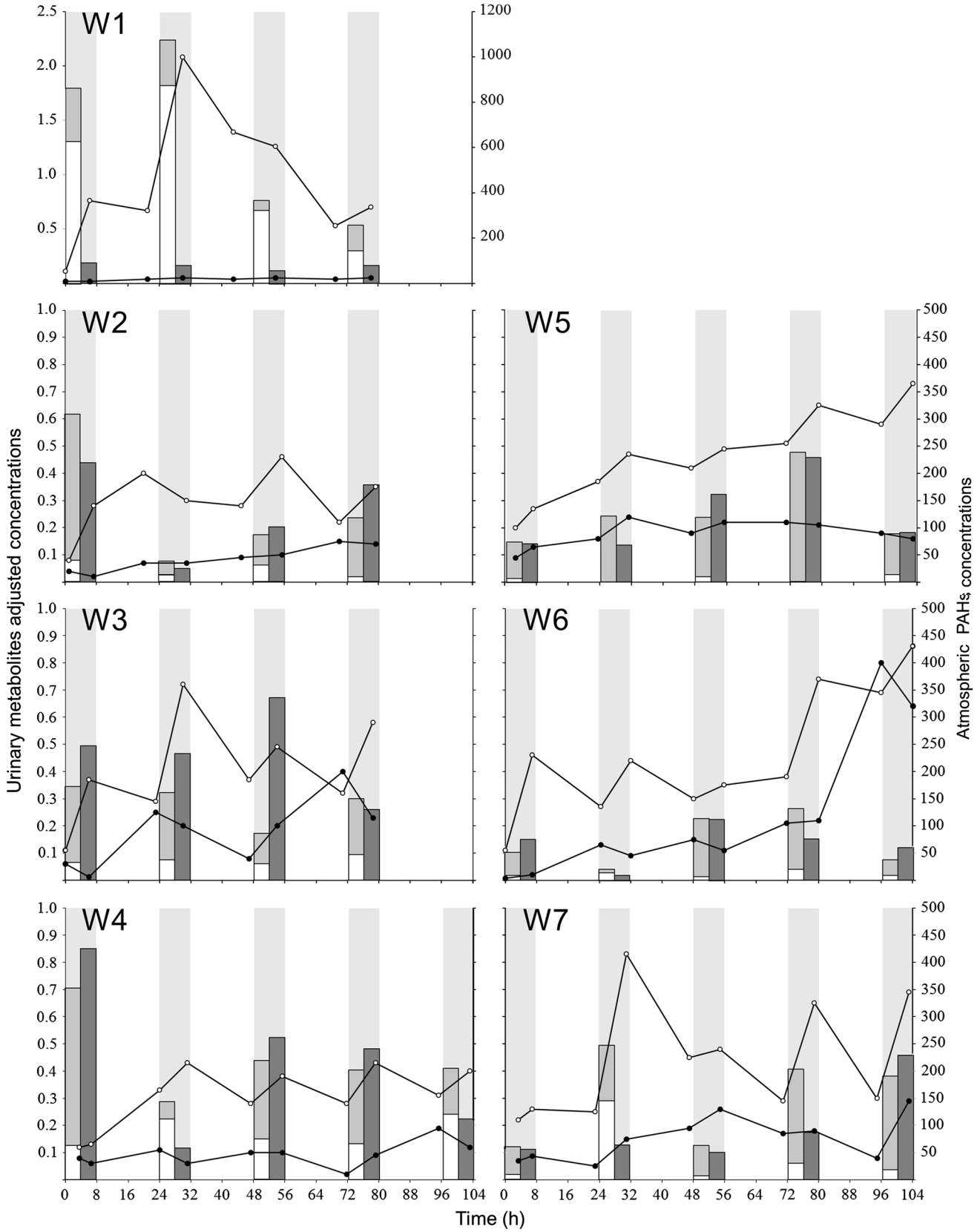

Fig. 1 Urinary concentrations of 3-OHBaP (nmol/mol) (*closed circles*) and 1-OHP (μmol/mol) (*open circles*) and daily atmospheric levels (ng/m^3) of gPyr (*white histograms*), pPyr (*light gray*) and BaP (*dark gray*) for every worker (W) during the working week. *Shaded zones* correspond to working hours

Table 4 Results from linear mixed effects models for 1-OHP

	1-OHP ES ($n = 32$)		1-OHP ES16 ($n = 25$)	
	Estimate (SE)	p value	Estimate (SE)	p value
Intercept	−0.793 (0.398)	0.058	−1.354 (0.361)	**0.002**
gPyr	0.114 (0.044)	**0.015**	−0.003 (0.040)	0.944
pPyr	0.086 (0.090)	0.346	0.197 (0.072)	**0.014**
1-OHP BS	0.554 (0.113)	**<0.001**	0.144 (0.137)	0.306
1-OHP ES			0.438 (0.150)	**0.010**

SE standard error

Significant p values are in bold

Table 5 Results from linear mixed effects models for 3-OHBaP

	3-OHBaP ES ($n = 32$)		3-OHBaP ES16 ($n = 25$)	
	Estimate (SE)	p value	Estimate (SE)	p value
Intercept	−0.798 (0.683)	0.254	−1.525 (1.001)	0.145
BaP	0.015 (0.132)	0.912	0.062 (0.188)	0.747
3-OHBaP BS	0.651 (0.117)	**<0.001**	0.051 (0.287)	0.861
3-OHBaP ES			0.309 (0.277)	0.279

SE standard error

Significant p values are in bold

especially for routine monitoring (Barbeau et al. 2011). The lower 3-OHBaP levels were observed at BSBW for all employees [<LOQ-0.09 nmol/mol] as well as for 1-OHP [0.08–0.22 μmol/mol] and were quite comparable to those reported in non-occupationally exposed subjects (Lafontaine et al. 2006). 3-OHBaP concentrations of mobile raw material dispatchers (W3) and safety rounds in the facility (W6) were above 0.4 nmol/mol, the recommended value proposed by Lafontaine et al. (2004), at BSEW. In contrast, 1-OHP levels were higher than 1 μmol/mol, as considered by some authors as the value that should not be exceeded (Siwinska et al. 2004), for the "carbon electrode extrusion" position only (W1). These differences between the two exposure biomarkers were confirmed by the high intra-individual variability of ratios 3-OHBaP/1-OHP. Previously, strong correlations had been found when the two metabolites were analyzed on the same sample collected at ESEW (Barbeau et al. 2014; Forster et al. 2008). These current results are explained by variable PAHs aerosol combined with respiratory uptake of gPyr through P3 respirators, and also by an important cutaneous absorption of particulate PAHs which can penetrate into the organism via unprotected wrist or neck, but also through skin areas covered by contaminated work clothes (Van Rooij et al. 1992). Indeed, it is well established that PAHs absorption process strongly influences the kinetics of urinary excretion of the various metabolites (Lafontaine et al. 2002; Li et al. 2012; Payan et al. 2009; Sobus et al. 2009b; St Helen et al. 2012; Viau et al. 1995; Viau and Vyskocil 1995).

Urinary levels of the two metabolites showed no progressive increase over the working week, except for W5 and W6, contrary to what was previously reported for 1-OHP in carbon electrode production (Petry et al. 1996). This can be explained by lower exposures in our study combined with high day-to-day variations. It is interesting to note that, during the first major occupational exposure of the working week, metabolite concentrations were high the following morning, while they came back to beginning of shift levels the other days of the week. This finding could probably be explained by the absence of accumulation of PAHs in organisms that were non-exposed since the previous weekend, promoting their distribution to different tissues and slowing their elimination. With regard to the end of the working week, 3-OHBaP concentrations were not significantly different between ES of the penultimate day and BSEW, unlike those of 1-OHP, as previously seen in 129 employees from different industrial sectors (Barbeau et al. 2014). However, in the current study, more than half (60 %) of the maximum urinary concentrations of 3-OHBaP were obtained at BSEW. The lack of 3-OHBaP accumulation in the organism from the fluctuating BaP exposure promotes the 3-OHBaP distribution to different tissues and slows its elimination, in the same way as the first working day with an occupational exposure. Thus, sampling for 3-OHBaP analysis should be carried out on BSEW as proposed by Gendre et al. (Gendre et al. 2004). On the other hand, the maximum levels of urinary 1-OHP always appeared at ES, but only 30 % were obtained at ESEW. In addition, this metabolite has a greater variation than 3-OHBaP due to faster elimination kinetics and simultaneous uptake of pPyr and gPyr through skin and respiratory tract, respectively. Significant positive effect of atmospheric gPyr levels on urinary 1-OHP concentrations on ES was shown by linear mixed effects models. Similar model was used to study effects of work assignments on the levels of PAHs in urines collected over three consecutive days in a group of workers exposed to hot asphalt (Sobus et al. 2009b). Thus, high levels of 1-OHP for W1 on the first and second days and for W7 on the second day are mainly due to atmospheric exposure to gPyr, and urine samples for the determination of 1-OHP should be taken at ES on the day of greatest exposure and not systematically at ESEW as previously suggested (Bouchard and Viau 1999). No significant effect of particular PAHs levels (BaP and pPyr) was observed on urinary metabolite concentrations. These results were similar to those previously obtained in a factory producing carbon electrodes (Bentsen et al. 1998). Indeed, the amount of 1-OHP eliminated in 24 h was not correlated with the level of atmospheric pPyr among employees producing creosote (Elovaara et al. 1995). These results are due to the wearing

of P3 mask that is designed to stop particulate phase (Rengasamy et al. 2009), and especially skin absorption of the compounds both by direct contact with the raw materials and through indirect contact via the particles deposited on work clothes and skin (Lafontaine et al. 2000). The importance of this absorption route delayed urinary excretion of metabolites due to PAHs skin retention (Sobus et al. 2009b; Viau et al. 1995; Viau and Vyskocil 1995). That is shown by significant effect of pPyr on urinary 1-OHP in ES16 samples. Sobus et al. (2009a) had also found that concentrations of 1-OHP measured in urine collected in the morning after the shift were related to dermal exposure to pyrene, measured using patches. As 3-OHBaP elimination is delayed by approximately 15 h compared with that of 1-OHP, as a result of kidney retention (Gendre et al. 2002, 2004; Marie et al. 2010), it is normal not to find any statistical link between urinary 3-OHBaP and atmospheric levels of BaP. The extent of dermal absorption of particulate PAHs restricts the relevance of proposing OEL for BaP and emphasizes the need for a biological limit value based on health effects and not derived from correlations with OEL.

Conclusions

This study demonstrates the advantage of biomonitoring for PAHs health risk assessment. Significant variations in the chemical composition of atmospheric aerosols and also different absorption pathways for gaseous and particulate PAHs preclude the use of 1-OHP instead of 3-OHBaP in order to assess exposure to PAHs carcinogenic mixtures. Although several authors have proposed an algorithm to adjust the urinary concentration of 1-OHP to the atmospheric pyrene/BaP ratio (Bouchard and Viau 1999; Jongeneelen 2014), this approach is of limited use. Indeed, it does not take into account either the differences between gPyr, pPyr and BaP absorption routes or urinary 1-OHP and 3OHBaP elimination kinetics. Urinary 3-OHBaP is the most relevant biomarker for estimating carcinogenic risk in subjects exposed to complex mixtures of PAHs. It has the advantage of assessing exposure to BaP, the only PAH classified as being clearly carcinogenic to humans. Furthermore, 3-OHBaP is more slowly eliminated in urine than 1-OHP, thus preventing underestimation of risk (Lin et al. 2005; Sobus et al. 2010). Urine samples should be collected at the beginning of the shift the end of working week for the 3-OHBaP quantification, especially in case of high exposure variability. Nevertheless, this study shows that the quantification of 1-OHP, in addition to 3OHBaP, in urine collected at different sampling times provides valuable information on absorption pathways and thus will contribute to improving the efficiency of protection measures.

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Conflict of interest The authors declare that they have no conflict of interest.

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