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REVIEW

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

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This article reviews the published studies on urinary 1-hydroxypyrene (1-OHP) as a biomarker of exposure to polycyclic aromatic hydrocarbons (PAHs) in work environments. Sampling and analysis strategies as well as a methodology for determining biological exposure indices (BEIs) of 1-OHP in urine for different work environments are proposed for the biological monitoring of occupational exposure to PAHs. Owing to the kinetics of absorption of pyrene by different exposure routes and excretion of 1-OHP in urine, in general, 1-OHP urinary excretion levels increase during the course of a work day, reaching maximum values 3–9 h after the end of work. When the contribution of dermal exposure is important, post-shift 1-OHP excretion can however be lower than pre-shift levels in the case where a worker has been exposed occupationally to PAHs on the day prior to sampling. In addition, 1-OHP excretion levels in either pre-shift, post-shift or evening samples increase during the course of a work-week, levelling off after three consecutive days of work. Consequently, ideally, for a first characterization of a work environment and for an indication of the major exposure route, considering a 5-day work-week (Monday to Friday), the best sampling strategy would be to collect all micturitions over 24 h starting on Monday morning. Alternatively, collection of pre-shift, post-shift and evening urine samples on the first day of the work-week and at the end of the work-week is recommended. For routine monitoring, pre-shift samples on Monday and post-shift samples on Friday should be collected when pulmonary exposure is the main route of exposure. On the other hand, pre-shift samples on Monday and Friday should be collected when the contribution of skin uptake is important. The difference between beginning and end of work-week excretion will give an indication of the average exposure over the work-week. Pre-shift samples on the first day of the work-week will indicate background values, and, hence, reflect general environment exposure and body burden of pyrene and/or its metabolites. On the other hand, since PAH profile can vary substantially in different work sites, a single BEI cannot apply to all workplaces. A simple equation was therefore developed to establish BEIs for workers exposed to PAHs in different work environments by using a BEI already established for a given work environment and by introducing a correction factor corresponding to the ratio of the airborne concentration of the sum of benzo(*a*)pyrene (BaP) equivalent to that of pyrene. The sum of BaP equivalent concentrations represents the sum of carcinogenic PAH concentrations expressed as BaP using toxic equivalent factors. Based on a previously estimated BEI of 2.3 μmol 1-OHP mol^{-1} creatinine for coke-oven workers, BEIs of 4.4, 8.0 and 9.8 μmol 1-OHP mol^{-1} creatinine were respectively calculated for vertical pin Söderberg workers, anode workers and pre-bake workers of aluminium plants and a BEI of 1.2 μmol 1-OHP mol^{-1} creatinine was estimated for iron foundry workers. This approach will allow the potential risk of cancer in individuals occupationally exposed to PAHs to be assessed better.

Keywords: polycyclic aromatic hydrocarbons, biological monitoring, urinary 1-hydroxypyrene, biological exposure indices.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous environmental contaminants, present as complex mixtures, and resulting from the incomplete combustion or pyrolysis of organic materials (Nikolaou *et al.* 1984, Lesage *et al.* 1987, Allaire *et al.* 1993, Petry *et al.* 1994). Many individual PAHs have been classified as possible or probable human carcinogens by the International Agency for Research on Cancer (IARC 1987). There is also sufficient evidence that workers employed in coke production, coal gasification, iron and steel founding and workers exposed to coal tar, coal tar pitches and soots are at higher risk of developing lung or skin cancer (Doll *et al.* 1972, Redmond *et al.* 1976, IARC 1984, 1985, 1987). There is also limited evidence of PAH carcinogenicity in workers in the aluminium production industry (IARC 1984). Furthermore, IARC has stated (1985) that there is sufficient evidence for the carcinogenicity of creosote in animals and limited evidence that coal tar-derived creosotes are carcinogenic to humans.

The most important source of PAH exposure is the work environment. The highest contents of PAHs were found in the coke industry and processes in which carbonaceous materials are produced or used, mostly at elevated temperatures (Björseth *et al.* 1981, Lindstedt and Sollenberg 1982). Lindstedt and Sollenberg (1982) have indeed classified work environments where exposure to PAHs can occur according to their levels of benzo(*a*)pyrene (BaP). Highest exposures (> 10 μg m^{-3}) were found in workers in gas and coke works, aluminium factories, manufacturing of carbon electrodes, those handling molten tar or pitch (roofing, paving, insulation coating), and those working in asphalting (when asphalt is mixed with tar).

Exposure in the work environment occurs mainly by inhalation (IARC 1984, ACGIH 1991) although the contribution of cutaneous exposure can be important (even predominant) in some types of work such as in aluminium works, in the petrochemical industry and in the handling of creosote (VanRooij *et al.* 1993b, Van-Rooij 1994, Boogaard and Van Sittert 1994). PAH in the atmosphere can be found either in the gaseous or particulate phase (Nikolaou *et al.* 1984, Lesage *et al.* 1987). Distribution between these two phases depends on the vapour pressure of the compound. The lower molecular weight PAHs (two- to four-ring PAHs) are found mainly in the gas phase while higher molecular weight compounds, such as BaP, are mostly found adsorbed to particles (Atkinson and Arey 1994).

Initially, monitoring of exposure to PAHs was conducted by analysis of airborne concentrations. Non-specific measurements for the estimation of the overall exposure to PAHs were performed by determining the amount of coal tar pitch volatiles (CTPVs) recovered as benzene-soluble matter (BSM) (OSHA 1976). This method does not, however, reveal the identity of PAHs present in the atmosphere. Other organic compounds present in the atmosphere can also contribute to the BSM. Chromatographic techniques for the individual identification and quantification of PAHs in these mixtures were then developed (Quilliam and Sim 1988, Hansen *et al.* 1991, Roussel *et al.* 1992, Wise *et al.* 1993). High performance liquid chromatography (HPLC) with fluorescence detection and capillary gas chromatography with mass spectrometry are among the most sensitive and selective methods (Law and Biscaya 1994). Moreover, NIOSH (1985) has recommended a standard procedure for the individual analysis of 16 major PAHs after separation by HPLC. PAH exposure is also often assessed using atmospheric BaP concentrations, owing to its carcinogenic potential.

Air monitoring only estimates the respiratory intake. However, as mentioned

previously, dermal exposure can occur in many work environments. Furthermore, adsorption of PAHs onto airborne particles can prevent their determination as BSM (Dufresne *et al.* 1987) and also alter their bioavailability and kinetics in the respiratory tract (Pelfrene 1976, Gerde *et al.* 1991). Therefore, biological monitoring appears as a useful complementary approach to assess workers' exposure to PAHs. Of course, a measurement of systemic exposure to PAHs does not have the same significance depending on the main route of exposure and hence a PAH exposure by the cutaneous route does not necessarily imply the same potential risk of lung cancer as an inhalation exposure.

Jongeneelen *et al.* (1986, 1987) proposed the measurement of urinary 1-hydroxypyrene (1-OHP) metabolite as a biomarker of the overall exposure to PAHs. The glucuronide of 1-hydroxypyrene is a major urinary metabolite of pyrene which is found in important amounts (1–10 %) in most PAH mixtures (Buchet *et al.* 1992, Roussel *et al.* 1992, Petry *et al.* 1994). Its method of determination is also sensitive and straightforward. Some researchers have measured urinary metabolites of other PAHs such as BaP, benzo(*a*)anthracene, naphthalene and phenanthrene (Jongeneelen *et al.* 1987, Grimmer *et al.* 1993, Hansen *et al.* 1994, Lintelmann *et al.* 1994, Popp *et al.* 1997). However, 1-OHP remains the most extensively studied.

This article presents an overview of the published studies on 1-OHP urinary excretion in workers and their contribution to the validation of this biomarker of PAH exposure, with emphasis on the methods of analysis and sampling strategies used. Biological monitoring strategies and an approach for determining biological exposure indices (BEIs) for various work environments are then proposed.

Analysis of 1-OHP in urine

Preservation of urine samples

In most studies, urine samples were usually immediately frozen and kept at –20 °C (Jongeneelen *et al.* 1985, 1987, Clonfero *et al.* 1989, Tolos *et al.* 1990, Boos *et al.* 1992, Burgaz *et al.* 1992, Granella and Clonfero 1993, Boogaard and Van Sittert 1994, Bouchard *et al.* 1994) or –80 °C (Whiton *et al.* 1995) until analysis. No other particular precautions were mentioned in most publications except that samples were kept in the dark (Granella and Clonfero 1993, Roos *et al.* 1997). In our laboratory, urine samples are collected over thymol to prevent bacterial growth (Bouchard *et al.* 1994). Quinlan *et al.* (1995c) mentioned that 1-OHP was stable for at least 3 days at 4 °C in urine adjusted to pH 5 and buffered. Quinlan *et al.* (1995b) also reported that 1-OHP in urine was as stable when matching samples were stored at 4°C or –20 °C until analysis and according to Boos *et al.* (1992), samples are stable for at least 6 months at –20 °C. Furthermore, samples are usually collected in polyethylene or polypropylene bottles or tubes (Becher and Björseth 1983, Clonfero *et al.* 1989, Tolos *et al.* 1990, Boos *et al.* 1992, Gardiner *et al.* 1992, Ny *et al.* 1993, Angerer *et al.* 1997, Roos *et al.* 1997).

Extraction and chromatographic analysis of urine samples

Most laboratories conducting analyses of 1-OHP in human urine have adopted the method of treatment and analysis described by Jongeneelen *et al.* (1985, 1987) or have made an adaptation of this method (tables 1 and 2). This simple and

sensitive method consists of the enzymatic hydrolysis of the glucurono- and sulpho-conjugated 1-OHP, solid phase extraction of the metabolite and HPLC analysis with fluorescence detection. The detection limit ranges from 0.1 to 1 nmol l^{-1} (Levin 1995). This HPLC method has, moreover, been recommended since 1990 for the biological monitoring of exposure to PAHs by the Working Group *Analyses in Biological Material* of the Commission for the Investigation of Health Hazards of Chemical Compounds in the work area (Jongeneelen and Anzion 1991).

Whiton *et al.* (1995) proposed a modification of the analytical approach proposed by Jongeneelen *et al.* (1985, 1987) to improve the method of quantification at very low levels. The modification aimed to correct for the matrix effect by quantifying, using a calibration standard addition to the methanolic extract of urine samples. On the other hand, Boos *et al.* (1992) and Lintelmann *et al.* (1994) developed a coupled-column HPLC method for the determination of 1-OHP by integrating the precolumn, thus sample processing, into the HPLC system, so reducing the analysis steps to enzymatic hydrolysis of urine samples, centrifugation and analysis of the pre-treated urines by HPLC with fluorescence detection.

A few authors have resorted to methods of treatment and analysis of pyrene metabolites different from the method of Jongeneelen and his colleagues. Grimmer *et al.* (1991, 1993) analysed 1-OHP urinary metabolites using a gas chromatographic technique. Conjugates were hydrolysed under acidic conditions (Grimmer *et al.* 1991) or enzymatically (Grimmer *et al.* 1993). 1-Hydroxypyrene was extracted from urine with toluene and methylated with diazomethane. The resulting methyl ether was purified by filtration over silica gel and chromatography on Sephadex LH20. Separation of the mixture was performed by gas chromatography (GC). Alternatively, Strickland *et al.* (1994) and Kang *et al.*(1995a, b) have recently developed a method to directly measure 1-OHP glucuronide by synchronous fluorescence spectroscopy (SFS) after immunoaffinity chromatography since in humans 1-OHP is excreted mostly as glucurono-conjugates which are approximately five-fold more fluorescent than free 1-OHP. Another method, used by Becher and Björseth (1983), consists of the reduction of PAH metabolites to unmetabolized forms and subsequent detection of the individual PAHs by HPLC/fluorescence. Recovery of pyrene and its metabolites is 36 %. It is also noteworthy that 1-OHP is usually expressed as a function of urinary creatinine to correct for variations in urine dilution.

Occupational studies on 1-OHP with emphasis on sampling strategies

Descriptive studies

Most of the published studies on 1-OHP urinary excretion in individuals occupationally exposed to PAHs are descriptive and concern the interindividual comparison of excretion values for the biological monitoring of exposure to PAHs or the assessment of the 1-OHP urinary excretion pattern with time to evaluate at what period spot samples are most likely to provide the best estimate of exposure.

Interindividual variations in 1-OHP urinary excretion and the effect of PAH sources other than occupational exposure

Several authors have compared the results of urinary 1-OHP measurements between exposed individuals of different work environments and control or

Table 1. Modifications brought by different authors to the treatment method of 1-hydroxypyrene in urine proposed by Jongeneelen *et al.* (1985, 1987).

Authors	Source of enzyme for hydrolysis	Amount of enzyme for hydrolysis (µl and/or units) per volume of urine sample (ml)	Incubation time (h)	Cartridge used for extraction of 1-OHP	Solvent for elution of 1-OHP from cartridge
Jongeneelen *et al.* (1985, 1987)	Boehringer Mannheim GmbH[a]	1250 units β-glucuronidase and 10 000 units arylsulphatase/25 ml	16	Sep-Pak C-18	Methanol
Tolos *et al.* (1990)	Sigma Chemical Co.[b]	7000 units β-glucuronidase and arylsulphatase/10 ml	4	Sep-Pak C-18	Methanol
Zhao *et al.* (1990)	Sigma Chemical Co.[c]	800 units β-glucuronidase and arylsulphatase/10 ml	1	Sep-Pak C-18	Methanol
Gardiner *et al.* (1992)	Not mentioned	7000 units β-glucuronidase and arylsulphatase/4 ml	4	Bond-Elut C-18	Methanol
Hansen *et al.* (1993)	Sigma Chemical Co.[d]	26 400 units β-glucuronidase and 440 units arylsulphatase/10 ml	20	Bond-Elut C-18	Acetonitrile
van Maanen *et al.* (1994)	Not mentioned	1250 units β-glucuronidase and 10 000 units arylsulphatase /25 ml	Overnight	Sep-Pak C-18	Methanol
Quinlan *et al.* (1995c)	Sigma Chemical Co.[e]	7000 units β-glucuronidase and arylsulphatase/4 ml	16	Bond-Elut C-18	Methanol
Hatjian *et al.* (1995)	Sigma Chemical Co.[c]	8000 units β-glucuronidase/20 ml	2	XL C-18 octadecyl	Methanol
Wu *et al.* (1998)	Sigma Chemical Co.[b]	2235 units β-glucuronidase and 82.5 units arylsulphatase/20 ml	4	Sep-Pak C-18	Methanol

[a] *Helix pomatia* solution with β-glucuronidase activity of 100 000 Fishman units per ml and arylsulphatase activity of 800 000 Roy units per ml.
[b] Type not mentioned.
[c] Type H1 from *Helix pomatia* with β-glucuronidase activity of 300 000–400 000 units per g of solid and arylsulphatase activity of 15 000–40 000 units per g of solid.
[d] Type HP-2 solution from *Helix pomatia* with β-glucuronidase activity of approximately 100 000 units per ml and arylsulphatase activity of up to 5000 units per ml.
[e] Type HP-2S solution from *Helix pomatia* with β-glucuronidase activity of approximately 100 000 units per ml and arylsulphatase activity of 1000–5000 units per ml.

Table 2. Modifications brought by different authors to the HPLC method of analysis of 1-hydroxypyrene proposed by Jongeneelen *et al.* (1985, 1987).

Authors	λ_{ex} and λ_{em}[a] (nm)	Mobile phase	Elution conditions
Jongeneelen *et al.* (1985, 1987)	242–388	MeOH:water[b]	Solvent gradient
Tolos *et al.* (1990)	244–390	ACN: water[c]	Solvent gradient[d]
Zhao *et al.* (1990)	345–388	MeOH: water	Solvent gradient[d]
Buchet *et al.* (1992)	244–390	ACN: water containing glacial acetic acid (0.5 ml/l)	Solvent gradient[d]
Gardiner *et al.* (1992) and Quinlan *et al.* (1995c)	240–388	MeOH: water	Isocratic elution: 75 % MeOH: 25 % water, flow rate of 1.1 ml min^{-1}
Hansen *et al.* (1993)	270–387	ACN: water	Isocratic elution in 10 min: 70 %ACN: 30 % water, flow rate of 1.0 ml min^{-1}
van Maanen *et al.* (1994)	237–384	MeOH: water	Solvent gradient[d]
Hatjian *et al.* (1995)	345–390	MeOH: water	Solvent gradient[d]
Roos *et al.* (1997)	240–390	ACN: water	Isocratic elution: 40 % ACN: 60 % H_2O water, flow rate of 1.0 ml min^{-1}
Wu *et al.* (1998)	241–388	ACN: water	Isocratic elution in 8 min: 65 %ACN: 35 % water, flow rate of 1.5 ml min^{-1}

[a] λ_{ex} and λ_{em} = excitation and emission wavelengths, respectively, for the fluorescence detection.
[b] MeOH:water = methanol:water.
[c] ACN:water = acetonitrile:water.
[d] Solvent gradient different from that of Jongeneelen *et al.* (1985, 1987).

reference groups with no known occupational exposure to PAHs (Jongeneelen *et al.* 1988c, 1990, Holland *et al.* 1984, Burgaz *et al.* 1992, Granella and Clonfero 1993, Vanhummelen *et al.* 1993, Viau *et al.* 1993, Ovrebo *et al.* 1994, 1995, Moen *et al.* 1996, Roos *et al.* 1997). Internal exposure levels between subjects of different industries were also compared (Göen *et al.* 1995, Levin 1995, Levin *et al.* 1995, Quinlan *et al.* 1995b, Zhao *et al.* 1995) and differences in 1-OHP urinary excretion in workers of different job categories at a same work site were established (Boogaard and Van Sittert 1995, Kang *et al.* 1995a, Levin *et al.* 1995, Quinlan *et al.* 1995b, Scheepers *et al.* 1995, Van Schooten *et al.* 1995). These studies were often conducted on single spot urine samples of workers exposed to PAHs (table 3). These spot samples were usually collected post-shift (end-of-shift) reflecting daily exposure including the contribution of exposure from previous days. Some researchers have, more specifically, measured post-shift samples at the end of a work-week (i.e. after at least 3 days of work), reflecting average exposure over the work-week. Other researchers have made single measurements of 1-OHP in individuals of various workplaces at another sampling time (second urine sample of the day at the end of a work-week or evening sample between 9 and 11 pm) or did not specify the time of sampling.

These studies showed that the concentrations were higher in individuals exposed to PAH in their work environment than reference or control populations, that excretion was influenced by factors such as smoking usually to a much smaller extent than occupational exposure, and that excretion of 1-OHP varied depending on the workplace and according to the job category (Jongeneelen *et al.* 1989, Zhao

et al. 1990, 1992b, Grimmer *et al.* 1991, Burgaz *et al.* 1992, Ovrebo *et al.* 1994, 1995, Clonfero *et al.* 1995, Göen *et al.* 1995, Levin *et al.* 1995, Omland *et al.* 1996, Angerer *et al.* 1997). A range of 1-OHP concentrations in different work environments and job categories and a range of values in the control populations have been established. Work environments and job categories have been classified from least to most at risk, according to the levels of 1-OHP in urine samples of workers. Levin (1995), in a report on the general conclusions and recommendations of the first international workshop on *1-OHP as a Biomarker for PAH Exposure in Man*, summarized the range of median urinary excretion of 1-OHP in workers from various workplaces. Coke-oven workers and those working in a graphite electrode production plant, a tar distillation plant, an aluminium smelter, and a creosote impregnation plant, showed, in decreasing order, the highest 1-OHP urinary excretion values. In glass manufacturing plant, chimney sweeping, iron foundry, meat smoke house, road paving, ground decontamination, car repair, waste incinerator and petrochemical plant workers, the range of median values of 1-OHP excretion was within that of unexposed individuals from different countries. Among the most exposed individuals, median 1-OHP excretion as high as 20 μmol mol^{-1} creatinine was observed in coke-oven workers which is much higher than the BEI of 2.3 μmol mol^{-1} creatinine proposed by Jongeneelen (1992, 1993) for this industry. Quinlan *et al.* (1995b) showed that mean 1-OHP excretion in coal liquefaction workers could reach values even higher than in coke-oven workers. In aluminium workers, some studies showed that 1-OHP levels can also exceed the BEI of 4.3 μmol mol^{-1} creatinine proposed by Ny *et al.* (1993) for this work site (Levin 1995, Van Schooten *et al.* 1995).

Furthermore, 1-OHP has been shown to be a valuable indicator of occupational exposure but also appeared sensitive enough to serve as an indicator of human exposure to low levels of PAHs provided the fraction of pyrene in the PAH mixtures has been established. Levin (1995) also reported median background concentrations of 1-OHP in urine samples of non-occupationally exposed individuals from various countries. Values ranged from 0.03 to 0.68 μmol mol^{-1} creatinine in non-smokers and from 0.07 to 0.76 μmol mol^{-1} creatinine in smokers depending on the country. Chénier and Viau (1997) showed that in a small group of eight male volunteers non-occupationally exposed to PAHs, intraindividual variability in 1-OHP urinary excretion was as important as interindividual variability.

In most reports, the influence of factors such as smoking, diet and alcohol was also investigated. It has been reported, in several studies, that in individuals with a low environmental exposure to PAHs, smoking normally caused a significant increase in urinary 1-OHP excretion (Jongeneelen *et al.* 1990, Granella and Clonfero 1993, Levin 1995, Levin *et al.* 1995, Viau *et al.* 1995a). However, as exposure levels increased, differences in 1-OHP excretion between smokers and non-smokers disappeared (Tolos *et al.* 1990, Buchet *et al.* 1992, Burgaz *et al.* 1992, Elovaara *et al.* 1995). Some authors have observed non-statistically significant increases in 1-OHP excretion in control smokers as compared with non-smokers (Jongeneelen *et al.* 1988b,c, Zhao *et al.* 1992a, Omland *et al.* 1994). Furthermore, Jongeneelen *et al.* (1990), Van Schooten *et al.* (1995) and Mielzynska *et al.* (1997) observed that the differences between the levels of 1-OHP in urine of smokers and non-smokers was more pronounced in the most exposed workers. These authors suggest a synergistic effect of smoking in combination with PAH exposure in the work environment on the excretion of 1-OHP in urine.

Table 3. Sampling strategies used in the published studies looking at 1-OHP urinary excretion in workers occupationally exposed to PAHs.

Number of urine samples	Determination of 1-OHP urinary excretion	Workers	Reference
Single spot	Post-shift (or end of work shift)	Coke and road workers	Grimmer *et al.* (1991)
		Workers of steel foundries (coke ovens and rolling mills) and graphite-electrode producing plant	Tas *et al.* (1994), Buchet *et al.* (1995)
		Workers of coke ovens and aluminium smelter and road pavers	Levin *et al.* (1995)
		Aluminium plant workers	Ovrebo *et al.* (1995)
		Workers of a graphite-electrode producing plant	Angerer *et al.* (1997)
		Cokery workers in an oil shale processing plant	Kuljukka *et al.* (1996, 1997, 1998)
		Iron foundry workers	Hemminki *et al.* (1997)
	Post-shift at the end of work-week	Workers handling petroleum coke	Jongeneelen *et al.* (1989)
		Workers exposed to bitumen fumes	Burgaz *et al.* (1992)
		Coke-oven workers	Clonfero *et al.* (1995)
		Coke-oven workers	Roos *et al.* (1997)
		Workers of a graphite electrode plant	dell'Omo *et al.* (1998)
	Second urine sample of the day at the end of a work-week	Iron foundry workers	Hansen *et al.* (1995)
		Iron foundry workers	Omland *et al.* (1996)
	Not specified	Coke-oven workers	Zhao *et al.* (1990)
		Bus and taxi drivers	Hemminki *et al.* (1994)
		Workers of an electrode paste plant	Ovrebo *et al.* (1994)
		Workers of carbon electrode production, road stone impregnation, aluminium smelting, glass manufacturing, chimney sweeping, meat smoking and municipal and industrial waste incineration	Göen *et al.* (1995)
		Workers of a steel plant	Kang *et al.* (1995a)
		Workers of engine rooms of ships	Moen *et al.* (1996)
	Between 9 and 11 pm	Coke-oven workers	Zhao *et al.* (1992b)
Two spot	Pre- and post-shift	Road asphalt-paving workers	Bos and Jongeneelen (1988)
		Road asphalt-paving workers, gate-keepers of a large harbour exposed to diesel exhaust gas	Jongeneelen *et al.* (1988b)
		Chip-sealing workers	Jongeneelen *et al.* (1988d)
		Workers of a coke production and graphite-electrode manufacturing plant	Buchet *et al.* (1992)
		Workers of a graphite-electrode producing plant and a coke-oven plant	Vanhummelen *et al.* (1993)
		Workers of coke and graphite-electrode producing plants	Ferreira *et al.* (1994)
		Coke-oven workers	Malkin *et al.* (1996)
		Coke-oven workers	Mielzyńska *et al.* (1997)
	Pre-shift at the beginning of work-week and post-shift at the end of the same work-week	Workers of a factory producing newspaper printing ink	Jongeneelen *et al.* (1988b)
		Workers of Söderberg pot room of an aluminium smelter	Ny *et al.* (1993)
		Coke oven workers	VanRooij *et al.* (1994a)
		Workers in petrochemical industries	Boogaard and Van Sittert (1995)
	Evening urine sample at the end of a work-week and pre-shift at the beginning of the following work-week	Automotive repair workers	Granella and Clonfero (1993)

	Second urinary voids at the beginning and end of the same work-week	Iron foundry workers	Hansen *et al.* (1994)
	Second urinary voids at the end of a work-week and at the beginning of the following work-week	Iron foundry workers	Omland *et al.* (1994)
Multiple spot	Pre- and post-shift on three consecutive work days	Coke oven workers	Bos and Jongeneelen (1988), Jongeneelen *et al.* (1990)
		Road-surfacing workers	Jongeneelen *et al.* (1988b, d)
		Pavers and roofers	Hatjian *et al.* (1995)
		Coke oven workers	Wu *et al.* (1998)
	Pre- and post-shift on five consecutive work days	Bake oven workers of a primary aluminium plant	VanRooij *et al.* (1992)
		Bake oven workers of an aluminium plant	Van Schooten *et al.* (1995)
		Workers of a carbon anode producing plant	Petry *et al.* (1996)
		Workers of a electrode paste producing plant	Bentsen *et al.* (1998)
	Pre- and post-shift during a week consisting of five work days and during two days off work	Workers of a creosote impregnation plant	Jongeneelen *et al.* (1988b)
		Coke oven workers	VanRooij *et al.* (1993a)
	Urine samples at the beginning and end of the work shift on the first, middle and last day of a 7-day work period	Workers of a coal tar distillation plant	Jongeneelen *et al.* (1986, 1988c)
	Pre- and post-shift urine samples on two alternate days	Aluminium workers from an anode bake area	Tolos *et al.* (1990)
	Overnight urine and urine voided between 10 and 16 h over a period of 10 days	Workers of a creosote impregnation plant	Bos and Jongeneelen (1988)
		Workers of a creosote impregnation plant	Jongeneelen *et al.* (1985, 1988c)
	Pre-shift urine samples on the first day of a work week and post-shift on every work day	Workers of a coke needle plant	Boogaard and Van Sittert (1994)
		Workers of petrochemical industries	Boogaard and Van Sittert (1995)
		Coal liquefaction workers	Quinlan *et al.* (1995c)
	Post-shift samples collected over the week	Workers of a carbon black manufacturing plant	Gardiner *et al.* (1992)
	Start-of-shift and end-of-shift urine samples as well as all samples voided during shift on four consecutive days	Coal liquefaction workers	Quinlan *et al.* (1995b)
	Start- and end-of-shift urine samples over four consecutive work weeks	Coal liquefaction workers	Quinlan *et al.* (1995 b)
	Once or twice-a-day during a weekend off work	Workers of a creosote impregnation plant	Jongeneelen *et al.* (1985, 1988b)
		Workers of a coke production and graphite-electrode producing plant	Buchet *et al.* (1992)
		Coal liquefaction workers	Quinlan *et al.* (1995b)
	Once-a-day during a 17-day period of vacation	Workers of a creosote impregnation plant	Jongeneelen *et al.* (1988b)
All urine voided	All urine voided from Sunday morning off work to Tuesday morning, Monday being a work day	Creosote workers of a wood impregnation plant	VanRooij *et al.* (1993b)
	All urine voided during the course of a work day for several work days including a week end off work	Workers of a creosote impregnation plant	Elovaara *et al.* (1995)
		Workers exposed to creosote-impregnated wood	Heikkilä *et al.* (1995)
24-h sample	24-h sample	Coke plant workers	Grimmer *et al.* (1993)
		Coke oven workers	Popp *et al.* (1997)
	24-h sample on the fourth or fifth work day	Workers of a carbon-electrode manufacturing plant	Van Delft *et al.* (1998)

It has also been observed that when environmental exposure to PAHs is low, the consumption of a PAH-rich diet can influence the excretion of 1-OHP (Buckley and Lioy 1992, Van Maanen *et al.* 1994, VanRooij *et al.* 1994b). However, when occupational or environmental exposure from other sources is important, the contribution of the diet to the overall exposure is negligible (Granella and Clonfero 1993). Finally, consumption of alcohol does not seem to have a significant effect on 1-OHP excretion in humans (Burgaz *et al.* 1992, VanRooij *et al.* 1994b, Van Schooten *et al.* 1995).

Patterns of 1-OHP urinary excretion with time

Some authors have examined 1-OHP urinary excretion at various time points. These studies allow the meaning of a measurement at different periods and the appropriate moment of sampling to be established. In several studies, urine samples were collected as two spot samples per individual (table 3). Internal exposure was often assessed by comparing pre- and post-shift (or beginning and end of work shift) 1-OHP urinary concentrations. This measurement has been shown to usually give an estimate of the daily exposure in the work environment taking background values into account, that is contribution of exposure from previous days. Alternatively, beginning and end of week urine samples were collected and allowed average weekly exposure to be evaluated provided that exposure is relatively constant over the work-week. In particular, either pre-shift samples at the beginning of the work-week and post-shift samples at the end of the same work-week were provided by workers or second urinary voids of the day at the beginning and end of the same work-week were obtained. Other sampling strategies included collection of the second urinary voids at the end of a work-week and at the beginning of the following work-week, or collection of urine samples in the evening at the end of a work-week and before shift at the beginning of the following work-week. Therefore, pre- and post-shift samples or beginning and end of week samples were compared showing that concentrations of 1-OHP varied during the work shift or during the course of the work-week. Values were, in general, mostly higher in samples collected at the end of the work shift than at the beginning of the shift and concentrations were higher at the end of the work-week than at the beginning of the work-week.

More detailed examination of 1-OHP urinary excretion pattern with time was also conducted from multiple spot urine samples in a same individual (table 3). Some researchers have collected pre- and post-shift (that is beginning and end of work) samples on three or five consecutive work days, or during a week consisting of five work days and two days off work. Other sampling strategies included collection of urine samples at the beginning and end of the work shift on the first, middle and last day of a 7-day work period; collection of pre- and post-shift urine samples on two alternate days; collection of overnight urine samples and urine voided between 10 and 16 h over a period of 10 days. Additionally, some researchers have collected pre-shift urine samples on the first day of a work-week and post-shift on every work day. Other types of multiple spot urine sample protocols included post-shift samples collected over the week, or start-of-shift and end-of-shift urine samples as well as all samples voided during shift on four consecutive days. In a few cases, multiple spot urine samples were collected over consecutive work-weeks. Urine samples have also been collected once or twice-a-

day during a weekend off work or during a long period of vacation. Finally, a few authors have collected all urine voided during the course of a work day, in some cases for several work days including weekends (table 3).

These studies, where several urine samples were collected per individual, have shown that 1-OHP excretion increased during the course of a work day reaching maximum values a few hours after the end of the shift. In most studies, it has been observed that 1-OHP levels in pre-shift samples were lower than in post-shift samples. Elovaara *et al.* (1995) have however reported that creosote workers of an impregnation plant, when exposed on the day prior to sampling, showed 1-OHP values mostly higher in the morning than at the end of the shift but 1-OHP values in evening samples were generally higher than in morning samples. This phenomenon was attributed to a probable continuing dermal absorption after working hours. These authors concluded that, when skin exposure is important, monitoring based on the urine sampling before and at the end of work may represent a rather meaningless sampling time for the assessment of pyrene exposure.

Furthermore, it has been shown that excretion values increased gradually over the first 3 days of the work-week but levelled off on the following work days (Jongeneelen *et al.* 1985a, 1988a, VanRooij *et al.* 1993a). 1-Hydroxypyrene excretion decreased during the weekend or a vacation of a few days, although excretion of 1-OHP in the most highly exposed individuals did not reach, upon return to work, the background levels of non-occupationally exposed individuals (Jongeneelen *et al.* 1985a, 1988a), suggesting a certain bioaccumulation of pyrene or 1-OHP in the body. Variations in week to week 1-OHP urinary excretion were also small (Quinlan *et al.* 1995b).

Some of the studies in occupationally exposed subjects also allowed a first order elimination half-life of 1-OHP in urine to be calculated. Jongeneelen *et al.* (1988b) observed two phases for the elimination of 1-OHP with half-lives of 1–2 days and 16 days in an operator worker of a creosote impregnation plant by collecting urine samples during a weekend off work and a period of vacation of 17 days, respectively. *A priori,* it would appear that the initial faster phase corresponded to the rapidly available part of the pyrene body-burden while the second slower phase was presumably linked to the elimination of pyrene stored in slowly perfused tissues such as adipose tissues. Values of 6–35 h, 13.4 h, 18 h and 19 h were respectively obtained for the initial phase of 1-OHP elimination in coke-oven workers (Jongeneelen *et al.* 1990) and those in the petrochemical industry (Boogaard and Van Sittert 1994), coke and graphite electrode manufacturing plants (Buchet *et al.* 1992), and a coal liquefaction plant (Quinlan *et al.* 1995b). These values are consistent with an increase in 1-OHP excretion over a work-week since they indicate that a worker repeatedly exposed to pyrene will not excrete all the 1-OHP produced between the end of a work day and the beginning of the following work day. Such an increase in 1-OHP urinary excretion values upon repeated exposure has indeed been observed in volunteers exposed to pyrene by the cutaneous route over five consecutive days (Viau and Vyskocil 1995).

Correlation between 1-OHP in urine and airborne PAHs and importance of dermal uptake

The airborne PAH profile from a given work site is relatively constant (Lesage *et al.* 1987). Studies have therefore attempted to evaluate the relationship between

airborne PAHs, expressed as benzene-soluble-matter (BSM), total PAHs, or individual PAHs such as BaP or pyrene, and 1-OHP urinary excretion. In general, they have been conducted using single spot urine samples (Levin *et al.* 1995, Zhao *et al.* 1990, 1992b) or two spot samples (Buchet *et al.* 1992, Ferreira *et al.* 1994, Hansen *et al.* 1994). A good correlation was obtained between PAH airborne concentrations and 1-OHP urinary excretion in several environments. Zhao *et al.* (1995) showed that concentrations of 1-OHP in human urine (1258 samples in 53 populations) correlated positively with airborne BaP concentrations at 53 locations ($r = 0.90$). Furthermore, some authors have evaluated dermal exposure to PAHs in several environments and have reported that its contribution to 1-OHP urinary excretion is significant, even predominant, in some workplaces.

Correlation between airborne concentrations of PAHs and 1-OHP in urine of coke-oven and aluminium workers

Table 4 shows that in several studies a significant correlation was established between airborne concentrations of either total PAHs, pyrene or BaP and 1-OHP urinary excretion measured as post-shift or evening levels, or change over the work shift or over a work-week in coke-oven and aluminium workers. It also appears that 1-OHP in urine is not only a good indicator of airborne exposure to pyrene but also to BaP and total PAHs in these work sites. Buchet *et al.* (1992) reported that the relationship between airborne pyrene concentrations and 1-OHP urinary excretion in coke-oven workers was not significantly different when expressing 1-OHP excretion as the change over the work shift ($n = 20$, $r = 0.74$, $p < 0.0001$) instead of post-shift levels ($n = 20$, $r = 0.76$, $p < 0.0001$). Levin *et al.* (1995), however, observed a poor correlation between airborne BaP concentrations and 1-OHP levels in post-shift urine samples in aluminium workers (potroom workers of a Söderberg plant). These authors reported that air measurements probably did not reflect the actual exposure since most of the workers wore respiratory protection in the potrooms.

Furthermore, a significant correlation was also observed between airborne concentrations of pyrene and total PAHs at coke plants (Jongeneelen *et al.* 1990, Buchet *et al.* 1992) and aluminium plants (Tolos *et al.* 1990, Ny *et al.* 1993), indicating that pyrene in air is a good indicator of airborne exposure to total PAHs in these work sites. The sum of airborne carcinogenic PAH concentrations (BaP, benzo(*a*)anthracene and dibenz(*a,h*)anthracene) was also highly correlated with pyrene in air at a coke plant ($n = 122$, $r = 0.83$, $p < 0.0001$) (Buchet *et al.* 1992).

Contribution of dermal exposure to the excretion of 1-OHP in urine

Some authors have attempted to establish the importance of dermal uptake on 1-OHP urinary excretion in workers from different work sites. Ferreira *et al.* (1994) observed that 1-OHP levels in post-shift urine samples of workers of a coke-producing and graphite electrode plant were statistically influenced by PAHs in air (by measuring 13 PAHs in the vapour and particulate phases) although the correlation coefficient was low ($n = 286$, $r = 0.5$). They noted that for a same external exposure to total PAHs as well as pyrene alone, 1-OHP urinary excretion values were higher in graphite electrode workers than in coke workers, suggesting a significant contribution of skin absorption in the former.

On the other hand, VanRooij *et al.* (1992) evaluated the importance of skin

Table 4. Correlation between airborne concentrations of PAHs in coke and aluminium plants and 1-OHP urinary excretion in workers.

Workers	Air monitoring	Determination of 1-OHP urinary excretion	Correlation coefficient (p)	Number of samples	Reference
Coke oven workers	Pyrene	End-of-shift after the third workshift of the week	0.40 ($p = 0.006$)	45	Jongeneelen *et al.* (1990)
	13 PAHs[a] (vapour and Particulate phases)	Post-shift	0.72 ($p < 0.0001$)	122	Buchet *et al.* (1992)
	Pyrene	Post-shift	0.67 ($p < 0.0001$)	122	
	Pyrene	Between 9 and 11 pm	0.65	21	Zhao *et al.* (1992b)
	Benzo(a)pyrene	Between 9 and 11 pm	0.69	21	
	13 PAHs (vapour and particulate phases)	Post-shift	0.50 ($p = 0.0001$)	286	Ferreira *et al.* (1994)
	Benzo(a)pyrene	Post-shift	0.69 or 0.77	20 or 17	Levin *et al.* (1995)
	Pyrene	Post-shift	0.56 ($p = 0.0002$)	41	Kuljukka *et al.* (1996, 1997)
	Benzo(a)pyrene	Post-shift	0.63 ($p = 0.00001$)	41	
	Benzene soluble fraction	Across-shift change (post-shift on the third work day minus pre-shift on the first work day)	0.70 ($p = 0.001$)	13	Wu *et al.* (1998)
	Benzene soluble fraction	Across-shift change (pre-shift on the fourth work day minus pre-shift on the first work day)	0.86 ($p = 0.0002$)	13	
Aluminium workers	Total PAHs (analysis of 17 individual PAHs using NIOSH method)	Change during shift	0.62 ($p = 0.006$)	18	Tolos *et al.* (1990)
	Pyrene	Change during shift	0.61 ($p = 0.0068$)	18	
	Coal tar pitch volatiles[b] or pyrene	Across-shift change (post-shift on the fifth work day minus pre-shift on the first work day)	0.84 ($p = 0.0001$)	28	Ny *et al.* (1993)
	Benzo(a)pyrene	Across-shift change (post-shift on the fifth work day minus pre-shift on the first work day)	0.79 ($p = 0.0001$)	28	
	Benzo(a)pyrene	Post-shift	0.31	9	Levin *et al.* (1995)

[a] Polycyclic aromatic hydrocarbons.
[b] Measured as the benzene soluble fraction.

contamination on the urinary excretion of 1-OHP in workers in the electrode production departments of pre-bake aluminium plants as compared with airborne exposure to PAHs. The correlation between skin contamination (wrist pad contamination) and post-shift 1-OHP urinary concentrations was higher than that observed between pyrene airborne concentrations and 1-OHP urinary excretion in post-shift samples although the difference was not significant ($n = 20$, $r = 0.40$ and 0.25, respectively). The correlation between pyrene air concentrations and 1-OHP urinary excretion was significantly higher when correlations were made using pre-shift urine samples of the day following air sampling than post-shift samples on the same day ($n = 20$, $r = 0.25$ and 0.47, respectively). It was therefore concluded that pyrene contamination on skin correlated equally or better with 1-OHP excretion than pyrene air concentrations. These authors further reported that the weekly increase in 1-OHP urinary excretion during a 5-day working period did not correlate well with pyrene air concentrations nor pyrene skin contamination (wrist pad contamination) ($n = 20$, $r = 0.18$ and 0.30, respectively). However, a better correlation was found between weekly 1-OHP urinary excretion and pyrene skin contamination than pyrene air concentrations.

VanRooij *et al.* (1993b) observed a good correlation between pyrene skin contamination (measurements on exposure pads) and urinary excretion of 1-OHP (difference between excretion with and without wearing coverall) in creosote workers of a wood preserving plant ($n = 10$, $r = 0.93$) whereas pyrene air concentrations were not well related to urinary 1-OHP ($n = 10$, $r = 0.39$). Elovaara *et al.* (1995) studied the relationship between 1-OHP levels in 24-h urine samples of creosote workers in an impregnation plant and airborne concentrations of pyrene but did not find any significant correlation ($n = 9$, $r = 0.34$). They reported that the contribution of inhaled pyrene was too small to explain the high daily urinary excretion of 1-OHP and suggested a probable contribution of dermal uptake. Jongeneelen *et al.* (1988d) also found a positive correlation between end-of-shift 1-OHP urinary excretion and pyrene on wrist pads ($n = 35$, $r = 0.36$) and hands ($n = 33$, $r = 0.63$) in chip-sealing workers. Furthermore, Quinlan *et al.* (1995b) calculated that dermal exposure contributed to more than 70 % of 1-OHP urinary excretion in coal liquefaction workers by comparing total excretion of 1-OHP in urine (area under the curve of 1-OHP urinary excretion rate versus time) with the contribution of airborne pyrene. Finally, Boogaard and Van Sittert (1994) reported that dermal and inhalation exposure were significant determinants of the 1-OHP urinary excretion in workers in the petrochemical industries and that the importance of dermal exposure varied according to the job category.

Relationship between 1-OHP urinary excretion and biological effects

In order to use urinary 1-OHP for the assessment of health risks due to PAH exposure, a quantitative relationship between the levels of this biomarker of exposure and toxic effects of PAHs should be established. Cross sectional epidemiological studies have evaluated the relationship between environmental exposure to PAHs and 1-OHP urinary excretion as seen previously (Zhao *et al.* 1990, 1992b, Buchet *et al.* 1992, Ny *et al.* 1993, Levin *et al.* 1995). Case-control and cohort studies have examined the relationship between environmental exposure to PAHs and the incidence of disease, in particular, lung and skin cancers (Lindstedt and Sollenberg 1982, Armstrong *et al.* 1994, Partanen and Boffetta 1994,

Ronneberg and Andersen 1995). However, molecular epidemiological studies attempting to establish a causal relationship between 1-OHP in urine and disease are lacking. Relationships between 1-OHP urinary excretion and early genotoxic effects (such as high frequency cells (HFC), sister chromatid exchanges (SCE) and micronuclei (MN)) have, nevertheless, been studied in humans. Some authors have also investigated the correlation between 1-OHP urinary excretion and biologically effective dose evaluated by measuring adducts formed between electrophilic metabolites of PAHs and DNA in white blood cells (WBCs), or blood proteins (such as haemoglobin and albumin) as surrogates for target tissue dose. One or two spot urine samples were usually collected in these studies (Vanhummelen *et al.* 1993, Hemminki *et al.* 1994, 1997, Tas *et al.* 1994, Buchet *et al.* 1995, Clonfero *et al.* 1995).

A significant positive correlation has been established between HFC or SCE in blood lymphocytes and 1-OHP excretion in urine of workers of steel foundries and graphite electrode producing plants (Buchet *et al.* 1995) and in coke-oven workers with a low exposure (Vanhummelen *et al.* 1993). It has also been reported that aromatic DNA adducts levels in white blood cells correlated with 1-OHP urinary excretion in post-shift samples collected from foundry workers (Hemminki *et al.* 1997) and cokery workers (Kuljukka *et al.* 1998). Van Schooten *et al.* (1995) found a highly significant correlation between the average PAH–DNA adduct values and the concentration of 1-OHP in the urine at the end of a 5-day work-week in smokers of a primary aluminium plant. This relationship was not, however, significant for non-smokers. These authors mentioned that the higher levels of PAH–DNA adducts observed in smokers could be attributed to the intake of PAH mixtures. These mixtures contain components that could enhance the metabolic pathway involved in the formation of electrophilic metabolites capable of binding to DNA. Correlations between PAH–DNA adducts in WBCs and urinary excretion of 1-OHP glucuronide have also been established in volunteers with no known occupational or medicinal exposure to PAHs after charbroiled meat consumption (Van Maanen *et al.* 1994, Kang *et al.* 1995b). These first authors suggested that the major biological determinants of the interindividual variations, such as absorption, metabolism and distribution, may be common to both indicators. On the other hand, Ovrebo *et al.* (1994) failed to observe any correlation between PAH–DNA adducts and 1-OHP urinary excretion in workers of an electrode paste plant. They noted, however, that 1-OHP excretion in urine reflects recent exposure while PAH–DNA adducts are more persistent and reflect the average exposure over some length of time.

Furthermore, Tas *et al.* (1994) established a weak but significant association between BaPdiolepoxide (BaPDE)–albumin adducts and 1-OHP excretion in post-shift urine samples from steel foundry and graphite electrode plant workers. Nielsen *et al.* (1996) have shown a strong relationship between hydroxyethyl valine haemoglobin adducts and 1-OHP urinary excretion in diesel-exhaust exposed workers but no correlation with DNA adducts levels. On the contrary, Omland *et al.* (1994) found no correlation between the levels of BaPDE–albumin adducts and 1-OHP urinary excretion, collected as the second voids on Friday and the following Monday, in iron foundry workers. These authors proposed that the lack of correlation could be due to differences in the PAH profiles between exposed and control groups, differences in the uptake, metabolism and excretion between individuals or that PAH concentrations found in these foundries were below the

threshold for increased BaPDE–albumin adduct formation. As mentioned for PAH–DNA adducts, it should be kept in mind that urinary 1-OHP reflects recent exposure whereas BaPDE–albumin adducts reflect the average exposure over a period of about 20 days, which corresponds to the half-life of human albumin. For steady-state exposure, a certain correlation between concentrations of 1-OHP in urine and adducts levels should however be apparent.

Proposal of biological monitoring strategies for 1-OHP in urine, reference values and a methodology for determining BEIs for various work environments

Analytical method

The straightforward HPLC method proposed by Jongeneelen *et al.* (1985, 1987) is a good basis for the analysis of 1-OHP in human urine. It is generally sensitive, specific and can be made very reproducible with some minor modifications (Bouchard *et al.* 1994). Alternative assays should be validated by comparison with this assay since quality of data and standardization of the analytical method are fundamental for interlaboratory comparison and interpretation of results. The HPLC method of analysis proposed by Jongeneelen *et al.* (1985, 1987) involves however the use of a solvent gradient and the run time is lengthy (50 min). To reduce the duration of chromatography, an isocratic elution can be used instead of a solvent gradient (see table 2). Furthermore, in a previous publication (Bouchard *et al.* 1994), we have shown that adsorption of 1-OHP on some LC-18 columns can occur. To improve reproducibility and sensitivity of the HPLC analysis, we suggest the addition of a very small concentration of ascorbic acid (1 mg l^{-1}) to the methanol eluent of the HPLC system. Indeed, it seems that ascorbic acid may act by masking specific adsorption sites – probably uncapped silica constituting the solid support for the hydrocarbon chains – that can retain 1-OHP.

In addition, since urinary levels of 1-OHP are dependent on urine output, 1-OHP urinary excretion values should be adjusted for creatinine levels. Urine should be collected in standard polyethylene or polypropylene tubes and we suggest the addition of a small amount of thymol to prevent bacterial growth. Tests conducted in our laboratory have further shown that storing of urine samples (collected over thymol) at room temperature, 4 °C or –20 °C causes no loss of the analyte for a period of several weeks (unpublished data). 1-Hydroxypyrene can also be preserved adsorbed on the Sep-Pak C-18 cartridge which allows the long distance transport of urine samples to be avoided. We have used this last approach in the frame of a study conducted in Central Africa (unpublished). Samples should however be placed at –20 °C when possible to maximize the length of preservation.

Sampling strategies

Since there are temporal changes in 1-OHP urinary excretion in workers exposed to PAHs, sampling time is critical for the evaluation of exposure data. It is therefore essential to have a proper knowledge of the urinary excretion kinetics of 1-OHP in order to establish the appropriate time of sampling and the significance of a measurement at different time periods. VanRooij *et al.* (1992) have also shown

that the relationship between 1-OHP urinary excretion and concentration of PAHs in the air and/or the skin varies depending on the sampling strategy. Empirical evidence and toxicokinetic considerations indicate that 1-OHP excretion increases during the course of a work day and highest values are consistently found in evening samples, that is 3–9 h after work. In many studies, a net increase in 1-OHP excretion within the work shift, by collecting pre- and post-shift urine samples, has been observed. However, in work sites where skin absorption of PAHs is important, it appears that post-shift 1-OHP excretion can be lower than pre-shift levels when workers have been exposed occupationally to PAHs on the day prior to sampling while 1-OHP excretion values are higher in evening samples than in pre-shift samples (Elovaara *et al.* 1995). When a worker has not been exposed occupationally to PAHs on the day prior to sampling, 1-OHP excretion values are however higher in post-shift samples than in pre-shift samples. It appears that if inhalation is the main route of PAH exposure, post-shift 1-OHP excretion values in all workers should consistently be higher than pre-shift values. On the other hand, if dermal contact is the main route of PAH exposure, following a weekend break, levels of 1-OHP in post-shift samples should be higher than in pre-shift samples. However, when exposed occupationally to PAHs on the day prior to sampling, 1-OHP excretion values in post-shift samples will most probably be lower than pre-shift levels while maximum values should be reached late in the evening. This is probably due to a slower absorption rate after dermal exposure than respiratory uptake, peak values being reached later in the evening in the former case than after inhalation exposure and is consistent with our observations in human volunteers (Viau and Vyskocil 1995). However, the first order elimination half-life of 1-OHP in urine for the initial faster phase is probably similar for the two exposure routes. Indeed, similar mean 1-OHP elimination half-lives were obtained in individuals of different work environments with varying potential for dermal uptake. Our group has furthermore observed a similar elimination half-life of 1-OHP in urine after oral and cutaneous exposure to pyrene in volunteers (Viau *et al.* 1995b). It can also be presumed that there is a continuing dermal absorption after working hours.

As a consequence of the previous remarks, when the contribution of dermal PAH exposure is important in a work environment, the difference between pre-shift/beginning of work-week and pre-shift/end of work-week 1-OHP urinary excretion will best reflect the average exposure over the work-week. On the other hand, when respiratory uptake is the main route of PAH exposure, the difference between pre-shift/beginning of work-week and post-shift/end of work-week 1-OHP urinary excretion will give the best indication of the average exposure over the work-week. It should be noted that the net average increase in 1-OHP excretion over a work day is often less than the change within a work-week and that pre-shift and post-shift or evening 1-OHP excretion values appear to level off, starting on the third consecutive day of the work-week. Furthermore, it appears that pre-shift values following several days away from work will indicate background values, that is general environment exposure and contribution of body burden in highly exposed individuals.

For a first characterization of a work environment and considering a 5-day work-week (Monday to Friday), the best sampling strategy would be to collect all micturitions over 24 h starting on Monday morning. Alternatively, pre-shift, post-shift and evening urine samples on Monday and Thursday or Friday should be collected. This will allow an indication of the predominant route of exposure to be

given and the most appropriate sampling strategy for routine monitoring to be established. As mentioned above, these sampling strategies will also allow daily exposure and average exposure over the work-week to be evaluated and subjects with a higher body burden to be identified. Furthermore, for routine monitoring of workers, when respiratory uptake is the main route of exposure, pre-shift sample on the first day of the work-week and post-shift urine sample on the last work day should be collected. On the other hand, when dermal uptake is important, collection of pre-shift samples on Monday and Friday is suggested.

Background values for non-exposed and exposed individuals

Mean background values are in the range 0.03 to 0.3 μmol 1-OHP mol^{-1} creatinine in non-smoking referents and 0.05 to 0.5 μmol mol^{-1} creatinine in smoking referents in the western Europe and Canada (Levin 1995). On the other hand, background values in workers, in other words, 1-OHP excretion following a period of a few days without exposure, are higher in the most exposed individuals such as coke-oven workers, aluminium workers, paving workers and coal liquefaction workers. There are also differences in 1-OHP baseline values from workers of different work environments as a consequence of the large variations in pyrene exposure dose, hence airborne concentrations and dermal uptake, from one workplace to another. Median background values of 2.79 and 1.64 μmol 1-OHP mol^{-1} creatinine have been reported in coal liquefaction workers after 2 and 3 days off work, respectively (Quinlan *et al.* 1995b). After a weekend break, mean pre-shift values of 13, 1.35 and 0.97 μmol 1-OHP mol^{-1} creatinine have respectively been observed in creosote workers, paving workers and highly exposed workers of an aluminium plant (Jongeneelen *et al.* 1988d, Elovaara *et al.* 1995, Van Schooten *et al.* 1995). Such a rise appears in agreement with the biphasic elimination of 1-OHP in urine observed in creosote workers (Jongeneelen *et al.* 1988b). Indeed, the slow phase, presumably corresponding to pyrene accumulation in secondary compartments from which it is slowly released, suggests that a worker continuously exposed to PAHs would show a progressive rise in background values of 1-OHP urinary excretion above those of non-occupationally exposed individuals until equilibrium between uptake and elimination is reached. However, results of a recent study in male Sprague–Dawley rats exposed to an acute dose of 50 μmol kg^{-1} of ^{14}C-pyrene showed that pyrene was rapidly distributed to lipids but elimination followed first order monophasic kinetics with a half-life of 4.9 h which suggest that long term accumulation of pyrene in fatty tissues of the rat is unlikely (Bouchard *et al.* 1998a). In addition, on average 82 % of the ^{14}C-pyrene dose was recovered in urine and faeces or present in the gastro intestinal tract 24 h after dosing. These results are also in accordance with those of Withey *et al.* (1991).

Methodology for determining BEIs for various PAH work environments

Airborne PAH profiles such as those of pyrene and known carcinogenic PAHs can vary substantially in different work environments. However, the relative distribution of PAHs in air samples from a certain work site is relatively constant. Differences in technological processes and different work temperatures can however lead to variations in PAH composition. Therefore, a single BEI for 1-OHP in urine is not applicable for all work environments. Jongeneelen (1992, 1993) and

Ny *et al.* (1993) have respectively proposed BEIs of 2.3 μmol 1-OHP mol^{-1} creatinine for coke-oven workers and 4.3 μmol mol^{-1} creatinine for Söderberg potroom workers of aluminium plants derived from the lower 1-OHP urinary excretion value corresponding to the TLV for CTPVs and BaP according to mathematical calculations. In other workplaces, there are insufficient data to calculate a BEI by such an approach. However, knowledge of the PAH profile, especially that of pyrene and carcinogenic PAHs, allows the introduction of a correction factor for these proposed BEIs by calculating the ratio of the sum of carcinogenic PAHs to pyrene airborne concentrations, where carcinogenic PAH concentrations are expressed as BaP equivalent concentrations using toxic equivalent factors (TEF). TEF have been estimated by some authors (Krewski *et al.* 1989, Collins *et al.* 1991). They were calculated by applying the same mathematical model of dose–response relationship to the data for each PAH compound and by comparing the results with those obtained for BaP. BaP is selected as the reference compound since it is one of the most potent carcinogens, and its carcinogenicity in several species and its mechanism of action are well documented. Multiplying the measured airborne concentrations of individual PAHs by their respective TEF allows the concentrations of the chemicals to be expressed in terms of BaP equivalents. BaP equivalent concentrations for each carcinogenic PAH are summed and the ratio of the sum of BaP equivalents to pyrene concentrations (ΣBaPequivalents/pyrene) can then be calculated to extrapolate results obtained from one work site to another. Therefore, using the TEF obtained by Krewski *et al.* (1989) and knowing the PAH profile of pyrene and carcinogenic PAHs in the work environments of interest, a ratio ΣBaPequivalents/pyrene was calculated. By applying this correction factor to the BEI proposed by Jongeneelen (1992, 1993) and provided that 1-OHP urinary excretion increases linearly with airborne pyrene concentrations, a tentative BEI was estimated for different work environments (see table 5) as follows:

$$BEI_w = BEI_c \frac{\left(\frac{\sum BaP_{eq}}{pyrene}\right)_c}{\left(\frac{\sum BaP_{eq}}{pyrene}\right)_w}.$$

where BEI_w = BEI in the work environment of interest

BEI_c = BEI proposed by Jongeneelen (1992, 1993) for coke-oven workers

$\left(\frac{\sum Bap_{eq}}{pyrene}\right)_c$ = Sum of BaP equivalents to pyrene airborne concentrations in the coke plant

$\left(\frac{\sum Bap_{eq}}{pyrene}\right)_w$ = Sum of BaP equivalents to pyrene airborne concentrations in the work environment of interest

Interestingly, using the PAH profile obtained by Björseth *et al.* (1978a,b, 1981) from stationary sampling in a vertical pin Söderberg plant and a coke plant and using the BEI proposed by Jongeneelen (1992, 1993) for coke-oven workers, the BEI calculated for vertical pin Söderberg plant workers using the proposed

Table 5. Ratio of the sum of BaP equivalent to pyrene airborne concentrations, biological exposure indices (BEIs) calculated for a few work sites and relative BEIs for various environments expressed according to the work site with the smallest BEI which is given an arbitrary value of 1.

Workplace		ΣBaPequivalent/pyrene[a] mean or mean ± SD[b]	Biological exposure indices[c] (μmol 1-OHP mol^{-1} creatinine)	Relative BEI values[d]	Reference for PAH profile
Coke plant		0.42	2.3	2	Björseth *et al.* (1978b)
		0.91 ± 0.18	1.1	1	Grimmer *et al.* (1993)
Aluminium plant	Vertical pin Söderberg	0.29	3.3	3	Björseth *et al.* (1981)
	Vertical pin Söderberg	0.22 ± 0.02	4.4	4	Björseth *et al.* (1978a)
	Anode production	0.12 ± 0.03	8.0	7	Björseth *et al.* (1978a)
	Pre-baked	0.099 ± 0.016	9.8	8	Björseth *et al.* (1978a)
Iron foundry		0.82	1.2	1	Knecht *et al.* (1986)

[a] The sum of BaP equivalent airborne concentrations was calculated as follows: (1) the mean airborne concentration of specific PAHs, for which a toxic equivalent factor TEF exists, was multiplied by the TEF reported by Krewski *et al.* (1989); (2) BaP equivalent airborne concentrations calculated for each PAH were summed; (3) the sum of BaP equivalent airborne concentrations were divided by pyrene airborne concentration.

[b] Depending on the available data.

[c] BEI for a work environment of interest is obtained using the sum of BaPequivalents to pyrene airborne concentrations in that work site and in coke plant, and using the BEI of 2.3 μmol 1-OHP mol^{-1} creatine proposed by Jongeneelen *et al.* (1992, 1993) for coke-oven workers. Airborne concentration values for coke plant are those reported by Björseth *et al.* (1978b).

[d] The different workplaces are expressed as a function of the work environment with the smallest BEI. They are calculated by assigning an arbitrary value of 1 to the workplace with the smallest BEI, and by using the respective BEI for each workplace which were determined according to the proposed approach.

approach (3.3–4.4 μmol 1-OHP mol^{-1} creatinine) is in the range of that proposed by Ny *et al.* (1993) (4.3 μmol mol^{-1} creatinine).

Furthermore, by attributing an arbitrary value of 1 to work site with the smallest BEI, work environments were classified according to their relative BEIs (table 5). The values obtained would mean that relative BEIs can vary up to eight times from one work environment to the other. It should however be kept in mind that a higher ΣBaP equivalents/pyrene ratio for a given workplace does not necessarily mean a higher cancer risk since the exposure dose has to be taken into account. Indeed, this simple approach indicates that considering equal atmospheric concentrations between two environments, the carcinogenic potential will be higher in the work environment showing the highest ΣBaPequivalents/pyrene ratio. Therefore, considering equal exposure concentrations between, for example, iron foundries and coke plants, the carcinogenic potential is higher in iron foundries than in coke plants. However, atmospheric concentrations are much lower in iron foundries than coke plants and thus the cancer risk should be smaller in the former work site.

It is noteworthy that, for iron foundry workers, the proposed BEI for 1-OHP urinary excretion is close to background levels of non-occupationally exposed individuals. This suggests that it could also be interesting to study urinary metabolites of other PAHs. For example, since PAH airborne concentrations vary between work sites, using a PAH metabolite other than 1-OHP, perhaps the difference between a proposed BEI for iron foundry workers and values in non-exposed subjects will be more important.

Obviously, these tentative BEIs rely on correlations between airborne BaP equivalent concentrations and urinary excretion of 1-OHP and consequently do not take into account that a fraction of 1-OHP urinary excretion results from cutaneous PAH exposure. Several studies have shown an important and, in some instances, major contribution of PAH skin exposure to 1-OHP urinary excretion in some workplaces, especially the graphite electrode industry, and in the electrode production departments of pre-bake aluminium plants, the petrochemical and coal liquefaction industries, and workplaces involved in the handling of creosote, as seen previously. Highest 1-OHP excretion values were moreover encountered in these work environments. Establishment of the composition of the PAHs in contact with skin and the rate of penetration of the various PAHs, and consideration of the relative contribution of inhalation and dermal exposures could allow a BEI to be calculated which took dermal exposure into account. Furthermore, although in humans only pulmonary exposure has yet been associated with lung cancer, both dermal and pulmonary exposure doses can account for pulmonary tissue dose (Schurdak and Randerath 1989, Withey *et al.* 1993). Therefore, dermal exposure could contribute to pulmonary cancer risk since, in rodents, it has been shown that PAH toxic effects is not restricted to the site of entry. Santodonato *et al.* (1981) have indeed observed the presence of pulmonary adenomas and leukaemia in mice exposed to BaP through the diet.

Finally, the proposed approach assumes that 1-OHP urinary excretion increases linearly with pyrene airborne concentrations which is a reasonable hypothesis based on experimental studies relating pyrene systemic doses to urinary excretion of 1-OHP (Bouchard and Viau 1998). It should however be noted that inter-individual variations in the absorption, metabolism, induction, distribution and excretion of PAHs, and the use of protective equipment can influence 1-OHP

excretion. Factors such as smoking and diet can also affect 1-OHP excretion levels although most researchers have observed that the contribution of smoking and diet appears negligible when PAH exposure levels in work environments are important. Therefore, between two subjects exposed to a same atmospheric pyrene concentration, 1-OHP excretion can vary substantially. Since PAHs are present as mixtures and are biotransformed by the P450-dependent mixed function oxidases, competition for or induction of the same metabolic pathway can occur. In the case of low-dose environmental exposure or when occupational exposure levels are maintained below the threshold limit value of 200 μg m^{-3} for CTPV in the air (ACGIH 1991), interference due to induction or competition is probably negligible based on experimental evidence (Bouchard *et al.* 1998b). However, when exposure levels are very high, depending on the PAH profile in a given environment, PAH enzyme induction or competition could occur respectively resulting in an increase or decrease in 1-OHP urinary excretion.

In conclusion, the basic method of analysis proposed by Jongeneelen and his colleagues should be adopted in all laboratories performing 1-OHP analysis for standardization of the data. Furthermore, the most appropriate 1-OHP urine sampling strategy for a first characterization of a work environment and considering a 5-day work-week appears to be collection of all micturitions over 24 h starting on Monday morning. Alternatively pre-shift, post-shift and evening urine samples on Monday and Thursday or Friday should be collected. For routine monitoring, pre-shift sample on Monday and post-shift sample on Friday should be collected when respiratory uptake is the main route of PAH exposure while collection of pre-shift samples on Monday and Friday is more appropriate when dermal uptake is significant. In addition, a methodology was developed for the determination of BEIs for different work environments using a BEI already established for a given work environment and taking into account the PAH airborne profiles in that work site and in the work site of interest. This approach also supports the BEI already proposed for aluminium Söderberg plants provided that the BEI proposed by Jongeneelen (1992, 1993) for coke oven workers is adequate. Until data from molecular epidemiological studies are available and the role of dermal exposure in the development of systemic cancers is better defined, or until a relationship between 1-OHP urinary excretion and PAH profile have been established for all work environments where PAH exposure can occur, this approach remains a useful tool for the establishment of BEIs for the biological monitoring of exposure to PAHs and for risk assessment.

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