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Benchmark Guideline for Urinary 1-Hydroxypyrene as Biomarker of Occupational Exposure to Polycyclic Aromatic Hydrocarbons

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Many individual polycyclic aromatic hydrocarbons (PAH) are genotoxic carcinogens. One of the parent PAH, pyrene, undergoes simple metabolism to 1-hydroxypyrene. 1-Hydroxypyrene and its glucuronide are excreted in urine. Biological monitoring of exposure to PAH has rapidly been expanded since urinary 1-hydroxypyrene was suggested as a biological index of dose of pyrene. Since pyrene is always present in PAH mixtures, the biological indicator is not only an indicator of uptake of pyrene, but also an indirect indicator of all PAH. At present, several hundreds of papers reporting on urinary concentrations of 1-hydroxypyrene in workers' urine are available. It appeared that urinary 1-hydroxypyrene is a sound biomarker and that the analytical method is robust and non-laborious. Since epidemiological studies of cancer mortality related to long-term average urinary 1-hydroxypyrene concentration are lacking, a sound health-based limit value of 1-hydroxypyrene in urine cannot be set as yet. Since PAH exposure is widespread and the dermal uptake is substantial among exposed workers, an attempt was made to propose a three-level benchmark guideline for urinary 1-hydroxypyrene. The reference value as a 95th percentile in non-occupational exposed controls is 0.24 μmol mol^{-1} creatinine and 0.76 μmol mol^{-1} creatinine for non-smokers and smokers, respectively. This is the first level of the benchmark guideline. A no-biological-effect-level of 1-hydroxypyrene in urine of exposed workers was found at 1.4 μmol mol^{-1} creatinine. It is the lowest reported level at which no genotoxic effects were found and therefore the estimate for the second level of the benchmark guideline. In two types of industry, cokeovens and primary aluminium production, the regression of airborne PAH concentrations and urinary 1-hydroxypyrene concentrations in exposed workers has been studied. The correlation of airborne concentrations and urinary 1-hydroxypyrene in urine of workers from cokeovens and in the primary aluminium industry was used to estimate the level of urinary 1-hydroxypyrene equal to the present occupational exposure limit (OEL) of PAH. The concentration of 1-hydroxypyrene in urine equal to the OEL is 2.3 μmol mol^{-1} creatinine and 4.9 μmol mol^{-1} creatinine, respectively, in these two industries. These latter values present the third level of the benchmark guideline. © **2001 British Occupational Hygiene Society. Published by Elsevier Science Ltd. All rights reserved**

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INTRODUCTION

The most important sources of occupational exposure to polycyclic aromatic hydrocarbons (PAH) are coal tars and derived products. Crude coal tar is a by-product of coke works and was in former times also a by-product of gasworks. Crude coal tar is usually distilled and blends of distillation fractions are used for various purposes: wood conservation, paints, road tars, roofing materials, etc. PAH concentrations in coal tar products may range up to approximately 10 wt%. A second source of PAH is petroleum distillates. However, heavy petroleum distillates contain much lower concentrations of PAH, namely in the parts-per-million range. A third source is the burning or pyrolysis of organic material at the workplace. Pyrolysis of organic material may result in PAH emission. Examples of this type of source are fire fumes, diesel exhaust gas, rubber fumes, waste incin-

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erator fumes, etc. PAH are present in the environment (air, water and soil) as trace contaminants. PAH are mostly non-volatile compounds. Airborne PAH with fewer than four aromatic rings (molecular weight range of 128–178) are merely present as gaseous compounds in the working environment. PAH with four rings (molecular weight of 202) are present both as gaseous compounds and as particulate matter. PAH with larger molecular weights (>228) are merely bound to airborne particulates.

The International Agency for Research on Cancer reported that workers from industrial settings where airborne PAH levels are high, such as gas works, coke works, and the primary aluminium industry, show excess rates of cancers. Aluminium production, coke production, coal gasification and coal tar pitches/coal tar fumes are carcinogenic to humans according to IARC classifications (IARC, 1983, 1984, 1985). PAH are genotoxic carcinogens, inducing chromosomal effects in exposed individuals. The most toxic effects on humans, other than cancer, arising from PAH-containing coal tars and creosote oils concern the skin and eyes. An overview of effects is given in the ACGIH's TLV documentation of coal tar pitch volatiles and benzo(*a*)pyrene (ACGIH, 1998).

Since the 1940s, the exposure of workers to PAH has been assessed by measurements of workroom air. In the 1970s personal air sampling replaced static air sampling. In many studies it has not been the PAH itself, but a surrogate, namely coal tar pitch volatiles (CTPV) as benzene soluble matter (BSM) which has been used as the indicator of airborne PAH. Only in the last decade has the direct determination of 16 EPA-PAH, or a single marker—that is benzo(*a*)pyrene [b(*a*)p]—in workroom air been more often chosen as the preferred marker. A recent change in the sampling method of PAH is that not only are the particulates sampled, but also the gaseous fraction of the PAH (Noto *et al*., 1996). The OEL for coal tar pitch volatiles of many countries is 0.2 mg m^{-3} as benzene soluble matter. Knowing that the US TLV of coal tar pitch volatiles (CTPV) has not been based on risk levels from epidemiological studies, but rather on analytical demands (detectable concentration of benzene solubles), it should be clear that the TLV of CTPV has no solid health-risk basement, but is more or less a practical compromise (ACGIH, 1998). The German TRK for benzo(*a*)pyrene is 2 µg m^{-3} (exception for cokeoven: 5 µg m^{-3}), which is a reasonably achievable limit. Several industrial processes emitting PAH (cokeoven, aluminium production, coal gasification, iron and steel founding) are classified as Class 1 carcinogens by the IARC (1984).

Studies of routes of exposure among PAH-exposed workers suggest that the major routes of occupational exposure to pyrene are through inhalation and cutaneous absorption. The dermal exposure of workers is very significant. It was estimated that the uptake of pyrene via the dermal route accounts for 50% of the total body dose for cokeoven workers (Vanrooij *et al*., 1993a). This is even as high as 90% for creosote-impregnating workers (Vanrooij *et al*., 1993b). Among coal liquefaction workers the dermal exposure was estimated to account for 70% of the excreted PAH metabolite (Quinlan *et al*., 1995). Another report shows that cokeoven workers show a substantial concentration of a PAH metabolite in urine even without detectable PAH in the air (Malkin *et al*., 1996). The excretion of 1-hydroxypyrene in urine of volunteers exposed to pyrene by the dermal route was very significant (Viau and Vyskocil, 1995).

There is a growing awareness that occupational PAH uptake via the skin is very substantial. Recently it became clear that dermal exposure to PAH not only results in local effects on the skin, but results also in systemic effects. PAH–DNA adducts were found in the lungs after dermal application of tar and bitumen products on the skin of mice (Schoket *et al*., 1988). Lung cancer risk following skin uptake of PAH can therefore not be ruled out.

The UK ACTS has agreed a framework for the use of biological monitoring guideline values as either health-based guidance values or hygiene-based guidance values, which are set around the 90th percentile of available validated data (HSE, 1996). Since no epidemiology with any biomarker as a dose indicator of PAH exposure is available, a health-based guideline value cannot be set. However, PAH form a class of genotoxic carcinogenic compounds for which exposure should be maintained at the lowest levels achievable. Therefore an attempt is made in this study to propose a benchmark guideline using the presently available knowledge of background exposure levels and exposure levels without genotoxic effects in PAH-exposed workers.

BIOLOGICAL MONITORING METHODS FOR PAH

Uptake of PAH in the body may be monitored by different biomarkers, for example metabolites in urine, urinary thioethers, urinary mutagenicity, PAH–protein adducts and PAH–DNA adducts. Both urinary mutagenicity and urinary thioethers are non-specific indicators of exposure to mutagenic agents. The latter two methods lack sensitivity in the case of occupational exposure to PAH and smoking is a very strong interfering confounder (Clonfero *et al*., 1989; Reuterwall *et al*., 1991; Ferreira *et al*., 1994a). These methods are not suitable for routine applications. Haemoglobin adducts of benzo(*a*)pyrene have been reported as possible biomarkers for exposure. However, there is still little experience and the first results show limited usefulness (Ferreira *et al*., 1994b).

A large effort has been made to use the extent to which PAH binds to DNA as a biomarker of exposure. White blood cells are used as surrogate target DNA. Enzyme immunoassays (Santella, 1988) and ^{32}P post-labelling assays (Beach and Gupta,

1992) have been suggested. Both methods may be subject to appreciable variation, as was demonstrated in several recent trials (Phillips and Castegnaro, 1993, 1999). A clear relationship between PAH exposure and PAH–DNA adducts has not yet been established. Moreover, the present post-labelling method is laborious.

A specific metabolite of pyrene, 1-hydroxypyrene, in urine has been suggested as a biomarker of human exposure to PAH (Jongeneelen *et al*., 1985). The abundance of the pyrene, a non-carcinogenic PAH, is relatively high in PAH mixtures (Jongeneelen *et al*., 1985; Buchet *et al*., 1992). Many reports from different sources confirmed the potential of the methodology (Jongeneelen *et al*., 1988, 1990, 1992; Clonfero *et al*., 1989; Tolos *et al*., 1990; Zhao *et al*., 1990, 1992, 1996; Buckley and Lioy, 1992; Burgaz *et al*., 1992; Gardiner *et al*., 1992; Sherson *et al*., 1992; Granella and Clonfero, 1993; Schaller *et al*., 1993; Tjoe Ny *et al*., 1993; Boogaard and van Sittert, 1994; Buchet *et al*., 1995; Levin, 1995; Quinlan *et al*., 1995; van Schooten *et al*., 1995; Gundel *et al*., 1996; Kuljukka *et al*., 1996, 1998; Malkin *et al*., 1996; Strickland *et al*., 1996; WHO, 1996; Angerer *et al*., 1997; Mielzynska *et al*., 1997; Popp *et al*., 1997; Merlo *et al*., 1998; Pan *et al*., 1998; Wu *et al*., 1998a; Cartensen *et al*., 1999). At present, urinary 1-hydroxypyrene is a widely used biological indicator of exposure to PAH; a search of the Toxline bibliographic database in 1999 with the keyword 'hydroxypyrene' resulted in approximately 400 papers reporting on studies of this biomarker. The conclusion of the first international workshop on 1-hydroxypyrene was that the analytical method is robust and that urinary 1-hydroxypyrene is a solid biological exposure indicator (Levin, 1995).

Recently, the glucuronide of 1-hydroxypyrene has also been used and proposed as an indicator of exposure, since the majority of 1-hydroxypyrene is conjugated and the fluorescence intensity is higher, but it has not yet shown its additional value (Strickland *et al*., 1996).

The measurement of various hydroxylated phenanthrenes has also been reported as a biomarker of exposure using GC-MS (Grimmer *et al*., 1991, 1993) and HPLC (Popp *et al*., 1997; Gundel *et al*., 1996). However, the experience is still limited and the sample clean-up procedure for the gas chromatographic analysis is more laborious. A recent attempt with immunoaffinity separation of PAH metabolites from urine of exposed workers showed the presence of both 1-hydroxypyrene and several hydroxyphenanthrenes (Bentsen-Farmen *et al*., 1999). A recent thorough review of biomarkers of PAH in environmental health suggests that urinary 1-hydroxypyrene as the most relevant parameter for estimating individual exposure to PAH (Dor *et al*., 1999).

1-HYDROXYPYRENE IN URINE

Metabolism and kinetics

Pyrene is metabolized into the intermediary 1-hydroxypyrene to form 1-hydroxypyrene–glucuronide, which is excreted (Jongeneelen *et al*., 1987). 1,2-Dihydroxy-1,2-dihydropyrene has been reported to be present in human urine as a minor metabolite (Grimmer *et al*., 1993). [^{14}C]Pyrene was rapidly distributed, metabolized, and eliminated from the body and 1-hydroxypyrene is a reliable indicator of systemic exposure to this polycyclic aromatic hydrocarbon. 1-Hydroxypyrene in urine indicates a constant fraction of total ^{14}C in urine and faeces (Bouchard *et al*., 1998).

The half-life for the urinary excretion of 1-hydroxypyrene in occupational exposed workers is 18 h (Buchet *et al*., 1992). The individual half-life ranges from 6 to 35 h (Jongeneelen *et al*., 1990). Boogaard and van Sittert (1994) reported a half-life of 13 (4–27) h in 16 workers. Therefore, urine should preferably be sampled end-of-shift at the end of the working week.

Biological levels without occupational exposure

Populations generally are exposed to traces of environmental PAH. Pathways include inhalation (air, smoking), ingestion (diet, drinking water) and dermal absorption (through medicinal drugs for the skin, children playing on and in dermal contact with contaminated soil). It is estimated that smoking and diet are the most important sources of PAH intake (IARC, 1983). Inhalation of urban air may be an additional source. Dermal treatment with pharmaceutical products containing low concentrations of tar or creosotes may be a significant source of dermally absorbed PAH. The conclusion of the first international workshop on 1-hydroxypyrene was that the baseline excretion value varies from country to country (Levin, 1995). Several studies among inhabitants of Europe, America and Asia show an increased level of urinary 1-hydroxypyrene among smokers; in most studies this level is significantly greater when compared with the levels for non-smokers (Table 1). Vanrooij *et al*. (1994) studied sources of inter-individual variation of urinary 1-hydroxypyrene. They concluded that smoking and dietary intake of PAH are the major factors contributing to the variation. Intake of polycyclic aromatic hydrocarbons is mainly from ingestion of dietary PAH and through smoking. Gundel *et al*. (1996) showed that the influence of smoking is of such an order of magnitude that potential environmental exposure to PAH is obscured by smoking.

Merlo *et al*. (1998) studied inter-individual differences in traffic police officers, including gene polymorphisms. The level of urinary 1-hydroxypyrene was clearly associated with cigarette smoking and to a lesser extent with exposure to outdoor PAH pol-

Table 1. 1-Hydroxypyrene in urine from non-occupationally exposed controls from various countries by smoking habits (Levin, 1995; WHO, 1996)

Country		1-Hydroxypyrene in urine (μmol mol^{-1} creatinine) as median (number of subjects)[a]	
		Non-smoking subjects	Smoking subjects
Netherlands	(1)	0.26 (52)	0.28 (38)
	(2)	0.17 (14)	0.51 (28)
Sweden		0.03 (48)	0.09 (10)
Turkey		0.24* (15)	0.33* (14)
Germany		0.04 (90)	0.12 (49)
Italy		0.08 (19)	0.13 (22)
Canada		0.07 (95)	0.12 (45)
USA	(1)	0.27 (10)	0.76 (11)
	(2)	0.44–0.61**	
	(3)	0.01–0.58***	
China		0.68* (74)	0.76* (84)

[a](*) Arithmetic mean; (**) arithmetic mean of four groups of 23 controls not split by smoking habits (Tolos *et al*., 1990); (***) range of five controls (Buckley and Lioy, 1992).

lution. A significant role of the cytochrome P450 1A1 and/or glutathione-S-tranferase M1 was not detected.

Recommended analytical method

The total of free and conjugated 1-hydroxypyrene is preferably determined with high pressure liquid chromatography (HPLC). After enzymatic hydrolysis to release the conjugated part of 1-hydroxypyrene the analyte is separated from the matrix and enriched by reversed phase column extraction. The components of the eluate are separated by HPLC and 1-hydroxypyrene is determined with a fluorescence detector. The results are corrected for urinary creatinine content.

The most comprehensive description of the method is that of Jongeneelen *et al*. (1987). Descriptions of other adapted methods include Boos *et al*. (1992) and Hansen *et al*. (1993). Reference to the independent testing of the methodcan be found in Angerer and Schaller (1991). Detection of 1-hydroxypyrene in urine is a toxicological analysis of biological material for which an European interlaboratory comparison programme has been established (Lehnert *et al*., 1999).

Sampling and storage

Urine should preferably be sampled at the end-of-shift on the last day of a routine working week. Sample contamination is not likely since 1-hydroxypyrene is a metabolite formed in the body. Urine can be collected in any clean container. Approximately 25 ml of urine will be sufficient for a duplicate analysis. Urine is collected in a container without preservative. When the samples are stored in the dark at 18°C, the samples can be kept for at least a year without loss of 1-hydroxypyrene (Jongeneelen *et al*., 1987).

JUSTIFICATION FOR A BENCHMARK GUIDELINE

Biological monitoring of occupational exposure to PAH has rapidly been expanded since urinary 1-hydroxypyrene was suggested in 1985 as a biological index of exposure to pyrene (Jongeneelen *et al*., 1985). Since pyrene is always present in PAH mixtures, the biological indicator is not only an indicator of uptake of pyrene, but also an indirect indicator of all PAH.

The composition of the PAH mixture in different work environments is, however, not fixed, but varies. When a single compound is used as an indicator or marker, one has to realize that the relative proportion of an individual PAH in the PAH mixture can vary from time to time and place to place. Comparative measurements of PAH in airborne particulate matter within a certain worksite have shown that the relative contribution of each PAH (the PAH profile) is relatively constant, but the relative proportion of pyrene in the PAH profiles between different worksites may differ significantly (König *et al*., 1985; Lesage *et al*., 1987). Since the relative proportion of pyrene in the PAH mixture derived from different work environments may vary, a biological exposure limit of 1-hydroxypyrene in urine valid for all industries cannot be set. Moreover, the epidemiological dose–response of urinary 1-hydroxypyrene and mortality is lacking, as with all new exposure indicators.

Polycyclic aromatic hydrocarbons represent a group of compounds with many genotoxic carcinogens; thus exposure should be maintained at as low a level as is reasonably possible. In occupational health practice there is a need for a guideline to interpret the levels of 1-hydroxypyrene in the urine of exposed workers. A benchmark guideline may fill this gap. There are several high-quality studies on exposure to PAH and their genotoxic effects in workers from different industries that can be used for the setting of some yardsticks.

Laboratory studies

In an animal model, the linearity of excretion of 1-hydroxypyrene was confirmed over an oral dose range of pyrene from 0.05 μg per rat to 500 μg per rat (Jongeneelen *et al*., 1985). The quantity of 1-hydroxypyrene in rat urine collected in 24 h samples during the 4 days after dosing, was proportional to the dose of pyrene over the whole range. [^{14}C]Pyrene was rapidly distributed, metabolized, and eliminated from

the body. 1-Hydroxypyrene in urine represented a constant fraction of total ^{14}C in urine and faeces (Bouchard *et al*., 1998).

Baseline levels

It is known that there is a baseline excretion of urinary 1-hydroxypyrene, mainly due to dietary intake of PAH and smoking (Table 1). With the distribution of urinary 1-hydroxypyrene in groups of referents, percentile values of background levels may be derived. Concentration of 1-hydroxypyrene in urine of male residents of a medium-sized city in The Netherlands (n = 100) expressed as a 95th percentile is 0.66 μmol mol^{-1} creatinine for non-smoking referents and 1.31 μmol mol^{-1} creatinine for smoking referents (Jongeneelen *et al*., 1988). Non-occupationally exposed males in The Netherlands (n = 76) had a 95th percentile of 0.29 μmol mol^{-1} creatinine and 0.79 μmol mol^{-1} creatinine for non-smokers and smokers, respectively (Vanrooij *et al*., 1994). Concentration of 1-hydroxypyrene in urine of female residents of an industrial area in Germany (n = 124), expressed as 95th percentiles, is 0.24 μmol mol^{-1} creatinine for non-smoking referents and 0.76 μmol mol^{-1} creatinine for smoking referents (Gundel *et al*., 1996). Zhao *et al*. (1996, abstract) estimated the limit levels of environmental PAH pollution. They proposed that 0.70 μg per 100 m^3 of benzo(*a*)pyrene for outdoor urban air and 0.34 μg $l.^{-1}$ of 1-hydroxypyrene in urine (equal to ≈0.70 μmol mol^{-1}) is the negligible risk level of urban residents in China.

The lowest reported 95th percentiles of non-occupational exposed individuals have been used to propose the first level of the benchmark guideline for 1-hydroxypyrene in urine; this first level is, for non-smokers, 0.24 μmol mol^{-1} creatinine and for smokers, 0.76 μmol mol^{-1} creatinine.

Field studies of the relation between urinary 1-hydroxypyrene and genotoxic effects in exposed workers

Three studies are available of genotoxic events in workers exposed to PAH which could be correlated to the level of urinary 1-hydroxypyrene.

1. Buchet *et al*. (1995) measured cytogenetic endpoints in lymphocytes, several tumour markers in serum and modified nucleosides in 149 workers. The Belgian workers were from cokeovens, rolling mills and from a graphite anode production plant, and were all heavily exposed to PAH with known levels of post-shift urinary 1-hydroxypyrene. The levels of the genotoxic effects were compared with the data of 137 controls. It appeared that sister chromatid exchanges and high-frequency cells (HFC–SCEs) in lymphocytes were the earliest biological effects and were consistently associated with the intensity of the current exposure to PAH. The level for urinary 1-hydroxypyrene in post-shift samples corresponding to 10% probability of increased HFC–SCEs was 6.0 μg g^{-1} (3.1 μmol mol^{-1}) for the non-smokers and 0.6 μg g^{-1} (0.3 μmol mol^{-1}) for the smokers. It was found that in the non-smoking workers an increased level of HFC–SCEs was not observed below the level of 2.7 μg g^{-1} (1.4 μmol mol^{-1}. This level is the *no-observed-effect-level* of this study.
2. German cokeoven workers (n = 29) exposed to an average benzo(*a*)pyrene concentration of 1.7 μg m^{-3} were studied for genotoxic effects (DNA single-strand breakage, DNA adducts and SCEs in lymphocytes). The cokeoven workers had significantly increased DNA-strand breaks in lymphocytes compared with standardized controls (n = 24). The workers had a mean 24 h excretion in urine of 3.6 μg 1-hydroxypyrene (47). A value of 3.6 μg 1-hydroxypyrene per 24 h urine equals on average 3.6 μg g^{-1} creatinine representing 1.9 μmol mol^{-1}. This level is the *lowest-observed-level* of genotoxic effects in this study (Popp *et al*., 1997).
3. Van Schooten *et al*. (1995) determined PAH–DNA adducts in lymphocytes of 57 exposed workers of a primary aluminium plant. The average adduct level of the high exposed group (number of workers equal to 29 from the bakeoven, anode factory and pot-relining departments) were significantly increased compared with a group of 14 controls. The average post-shift urinary 1-hydroxypyrene level of this high exposed group was 3.8 μmol mol^{-1} for non-smokers and 7.2 μmol mol^{-1} for smokers. The level of 3.8 μmol mol^{-1} is the *lowest-observed-level* of a specific genotoxic effect.

Table 2 summarizes the data of the three studies. There is also a number of studies of PAH–DNA adducts in aluminium and electrode paste plant workers that are negative (Cartensen *et al*., 1999). The study of Buchet *et al*. (1995) showed a no-observed-effect-level in non-smoking workers of genotoxic effect markers at a level of 1.4 μmol mol^{-1} creatinine. It seems to be the lowest exposure level in PAH-exposed cokeoven plus graphite anode workers without genotoxic effects. This level is proposed as the second level of the benchmark guideline.

Field studies of the relation between personal airborne PAH and urinary 1-hydroxypyrene in exposed workers

Further, a third level of the benchmark guideline can be proposed, based on studies with air concentrations of PAH and urinary 1-hydroxypyrene. Individual authors have reported on the relation of airborne PAH and 1-hydroxypyrene in urine among workers. The airborne occupational exposure limit (OEL) of PAH is expressed as BSM equal to 0.2 mg m^{-3} (TLV from USA) or as benzo(*a*)pyrene equal to

Table 2. Studies of PAH-exposed workers in which genotoxic effects were found with urinary 1-hydroxypyrene as exposure indicator

1-Hydroxypyrene in end-of-shift urine sample (μmol mol^{-1})	As derived from:	Reference
1.9[a]	Urine concentration in 24 h urine samples in cokeoven workers with increased single-strand breaks in lymphocytes = *Lowest-Observed-Effect-Level*.	Popp *et al*., 1997
1.4	End-of-shift urine concentration with no increase of the most sensitive (HFC-SCEs) of many BEM markers in cokeoven workers plus graphite anode plant workers = *No-Observed-Effect-Level*.	Buchet *et al*., 1995; Lauwerys, 1997
3.8	End-of-shift urine concentration with an increase of DNA adducts in lymphocytes of primary aluminium workers = *Lowest-Observed-Effect-Level*.	van Schooten *et al*., 1995

[a]Derived as creatinine-corrected concentration from a concentration of 3.6 μg in 24 h urine samples.

2 μg m^{-3} (TRK from Germany). Four studies are available in which a recommendation for a urinary limit value equal to the present available OEL is made, and four studies present data to derive a limit value. These studies are described below.

1. Jongeneelen *et al*. (1990, 1992)studied the PAH exposure of 56 cokeoven workers from The Netherlands. They did personal 8 h air sampling and at the end of the working week urine samples were collected. Total PAH and benzene soluble matter (BSM) were determined in the air samples. Urine samples were tested for 1-hydroxypyrene. A regression line of the concentration BSM in the air and end-of-week urinary 1-hydroxypyrene was calculated in order to find the concentration of 1-hydroxypyrene equalling the TWA–TLV of 0.2 mg m^{-3} BSM. Doing this they proposed an end-of-working-week biological exposure limit of 2.3 μmol mol^{-1}.
2. Vanrooij *et al*. (1993a) followed 12 cokeoven workers of a second Dutch cokeoven during a working period of five shifts. During every shift 8 h personal air sampling of PAH was done and end-of-shift urine samples were collected. A regression equation of the five-shift average concentration of benzo(*a*)pyrene in the air and end-of-week urinary 1-hydroxypyrene was calculated. These authors proposed a limit of 3.2 μmol mol^{-1} (end of working week) as equal to an airborne concentration of 2 μg m^{-3} benzo(*a*)pyrene (equivalent to the German TRK value).
3. Kuljukka *et al*. (1996) studied the exposure of 49 Estonian cokery workers to PAH at a shale oil processing plant. Two personal 8 h air samples of PAH and two urine samples at the end of shift for each worker were collected. Totals of PAH were determined in the air samples. Urine samples were tested for 1-hydroxypyrene. A regression equation of the concentration of benzo(*a*)pyrene in air and the increase of urinary 1-hydroxypyrene over the working week was derived from the data of the authors, after supplying additional data (personal communication). The data showed that at an airborne concentration of 2 μg m^{-3} b(*a*)p (equivalent to the German TRK value) equals an end-of-shift urinary 1-hydroxypyrene level of 3.0 μmol mol^{-1}. The mean PAH–DNA adduct level in white blood cells (WBC) of these cokeoven workers was not significantly increased, compared with controls (Kuljukka *et al*., 1998).
4. In a group of 24 Polish cokeoven workers, the median airborne b(*a*)p concentration varied by 2.1 μg m^{-3} and the median concentration of 1-hydroxypyrene in end-of-shift samples was 2.26 μmol mol^{-1}. An estimation of a limit value was not made by the authors, probably because of the low correlation of b(*a*)p in air and 1-hydroxypyrene in urine (r = 0.26) (Mielzynska *et al*., 1997). An explanation might be that the existing dermal exposure varies strongly between workers. On average it seems that at an airborne concentration of 2 μg m^{-3} b(*a*)p (equivalent to the German TRK value) in this study represents an end-of-shift urinary 1-hydroxypyrene level of 2.3 μmol mol^{-1}.
5. Wu *et al*. (1998a) studied the individual occupational exposure of 80 Taiwanese cokeoven workers to benzene soluble fraction (BSF) and the resultant relation to urinary 1-hydroxypyrene. Individual personal air samples of BSF and pre-shift and post-shift urine samples were obtained. The regression model showed a high correlation of post-shift urinary 1-hydroxypyrene with individual BSF in the air (r = 0.74, n = 80). The plot of the regression equation of the BSF concentration in air and post-shift urinary 1-hydroxypyrene showed that at an airborne concentration of the TWA–TLV of 0.2 mg m^{-3} BSF and an end-of-shift urinary 1-hydroxypyrene level of approximately 48.0 μg g^{-1} (equal to 25.0 μmol mol^{-1} creatinine) are found.

6. Pan *et al*. (1998) studied multiple biomarkers for individual occupational exposure in Chinese cokeoven workers. Personal air samples and end-of-shift urine samples were obtained from 75 workers. The plot of the regression equation of the total concentration of PAH in the air and post-shift urinary 1-hydroxypyrene showed a correlation coefficient of 0.78. Workers with an average exposure to benzo(*a*)pyrene of 2.0 μg m^{-3} (the German TRK value) showed an average end-of-shift concentration of 9.1 μmol mol^{-1} creatinine.
7. Tjoe Ny *et al*. (1993) performed industrial hygiene measurements of PAH amongst 38 workers in a Söderberg potroom (primary aluminium production) in Surinam (South America). One personal 8 h air sample of PAH and one post-shift urine sample on the first day and one sample at the end-of-shift on the last day of the working week for each worker were collected. Coal tar pitch volatiles as benzene solubles (CTPV) were determined in the air samples. Urine samples were tested for 1-hydroxypyrene. A regression equation of the CTPV concentrations in air and increase of urinary 1-hydroxypyrene over the working week was calculated in order to find the concentration of 1-hydroxypyrenene equalling the TWA–TLV of 0.2 mg m^{-3} CTPV. They found 4.3 μmol mol^{-1} as the TLV-equal urinary concentration, expressed as a maximal weekly increase. The mean pre-working week concentration of 1-hydroxypyrene was 0.6 μmol mol^{-1}. The TLV-equal concentration of 1-hydroxypyrene for workers in primary aluminium production, expressed as an end-of-shift limit value, is 4.3 + 0.6 = 4.9 μmol mol^{-1}.
8. Angerer *et al*. (1997) reported industrial hygiene measurement of PAH among 67 workers in a graphite electrode producing plant in Germany. A personal 8 h air sample of PAH and one urine sample after the working shift of each worker were collected. Benzo(*a*)pyrene was determined in the air samples. Urine samples were tested for 1-hydroxypyrene. A regression equation of the concentration of benzo(*a*)pyrene in the air and 1-hydroxypyrene in post-shift urine was calculated in order to find the concentration of 1-hydroxypyrenene equalling the German limit value (TRK) of 2 μg m^{-3} benzo(*a*)pyrene. They suggested 40 μg g^{-1} creatinine (95%CI range of 20–74). This is equal to 21 μmol mol^{-1} creatinine.

The data are summarized in Table 3. The data were obtained from two types of industry. Since it is known that the PAH profile can vary substantially in different worksites and industries, a third level of the benchmark guideline was suggested for each of the two types of industry:

- *Cokeoven*: an end-of-working week value of 2.3 μmol mol^{-1} creatinine is the lowest reported estimate for the concentration equal to the OEL. This concentration of 2.3 μmol mol^{-1} creatinine is proposed as the best available estimate for the third level of the benchmark guideline, solely valid for cokeoven workers.
- *Primary aluminium industry*: an end-of-working week value of 4.9 μmol mol^{-1} creatinine is the lowest estimate for the concentration equal to the OEL from two studies. It is known that the relative proportion of pyrene in the PAH mixture is not constant; the CTPV of the primary aluminium industry contains relatively more pyrene than the CTPV of cokeovens. The urine concentration in primary aluminium workers will therefore be somewhat higher when reaching the CTPV limit value. The concentration of 4.9 μmol mol^{-1} creatinine is proposed as the best available estimate of the third level of a benchmark guideline, solely valid for workers involved in primary aluminium production and graphite electrode production.

DISCUSSION

PAH-exposed workers undergo inhalation exposure, dermal exposure and oral exposure to a certain extent. Any exposure to genotoxic carcinogens as represented by PAH mixtures is assumed to pose a certain excess risk of cancer.

The concentration of 1-hydroxypyrene in urine, collected at the end of a working week, can be used as a biological indicator of recent doses of PAH. The presence of 1-hydroxypyrene in the urine represents the sum of resorption in the airways, resorption in the gastro-intestinal tract due to the swallowing of coarse particles and via the dermal route. The urine test is specific for exposure to pyrene, which is always present in mixtures of PAH. At present, 1-hydroxypyrene in urine is often used as a biomarker of the dosage of PAH. Pyrene itself is not genotoxic, but the PAH mixture contains many other genotoxic PAH.

The relative proportion of pyrene in the PAH mixtures from different industries or worksites may vary. This implies that the ratio of pyrene to carcinogenic PAH also varies. This factor is an important confounder of the dose–response relationship of the long-term average of urinary 1-hydroxypyrene versus excess cancer in exposed workers. The variation of the pyrene content in the total PAH for the various work environments makes obligatory the analysis of the abundance of pyrene in the PAH mixture at each site where workers are being monitored for urinary 1-hydroxypyrene.

No epidemiology of the long-term average of urinary 1-hydroxypyrene versus cancer outcome is available as yet. A sound health-based limit value, valid for all types of industries, cannot be recommended at present. An approach based on benchmark guidelines is the best feasible alternative for the moment. Three

Table 3. Studies of personal air sampling of PAH and 1-hydroxypyrene in urine of workers used for an estimation of the urinary metabolite level equal to the airborne occupational exposure limit of PAH

1-Hydroxypyrene in end-of-shift urine sample (μmol mol^{-1})	Type of industry	As derived from:	Reference
2.3	Cokeoven	End-of-shift concentration in urine at 0.2 mg m^{-3} BSM in Dutch cokeoven workers (first plant).	Jongeneelen, 1992
3.2	Cokeoven	End-of-shift concentration in urine in Dutch cokeoven workers at 2 μg m^{-3} b(*a*)p (second plant).	Vanrooij *et al*., 1993a
3.0	Cokeoven	End-of-shift concentration in urine at 2 μg m^{-3} b(*a*)p (=TRK–G) in oil shale Estonian cokery workers.	Kuljukka *et al*., 1996
2.26	Cokeoven	Median end-of-shift concentration in urine at a median airborne concentration of 2.1 μg m^{-3} b(*a*)p in Polish cokeoven workers.	Mielzynska *et al*., 1997
25.0	Cokeoven	End-of-shift urine concentration at 0.2 mg m^{-3} BSM in Taiwan cokeoven workers.	Wu *et al*., 1998a
9.1	Cokeoven	End-of-shift urine concentration at 2 μg m^{-3} benzo(*a*)pyrene in Chinese cokeoven workers.	Pan *et al*., 1998
4.9	Primary aluminium production	End-of-shift urine concentration at 0.2 mg m^{-3} BSM in workers of a Söderberg aluminium plant in Surinam	Tjoe Ny *et al*., 1993
21.0	Primary aluminium production	End-of-shift urine concentration at 2 μg m^{-3} b(*a*)p in German graphite electrode production workers.	Angerer *et al*., 1997

levels are proposed. The first level of the benchmark guideline is derived from the consideration that PAH mixtures are carcinogenic to humans, acting via a genotoxic mechanism. Exposure to the PAH mixture should be modified to the lowest feasible level. There is a baseline excretion of urinary 1-hydroxypyrene in referents, mainly due to dietary intake of PAH and smoking. The 95th percentile of referents are 0.24 μmol mol^{-1} creatinine for non-smokers and 0.76 μmol mol^{-1} creatinine for smokers. This is proposed as the first level of the benchmark guideline.

The second level is the prevention of genotoxic events such as DNA adducts and chromosomal effects in exposed workers. The long-term significance of these genotoxic effects is still unknown. For preventive action it seems to justified to maintain the exposure at a level which prevents an increased prevalence of genotoxic effects. The no-observed-effect-level of genotoxic effects in workers is found at a concentration level for 1-hydroxypyrene of 1.4 μmol mol^{-1} creatinine.

A third level of the benchmark guideline can be set at the level of the present OELs for PAH. However, the relative proportion of pyrene in the PAH mixture derived from different industries or worksites is not constant and this confounds the relationship between airborne PAH and urinary 1-hydroxypyrene in exposed workers. It seems that the PAH mixture is constant within a certain type of industry; therefore an industry-specific benchmark guideline is proposed. For cokeovens and primary aluminium production several studies are available in which the concentration for airborne PAH is related to urinary 1-hydroxypyrene. The OEL equal levels of urinary 1-hydroxypyrene in cokeoven workers show a wide range; the concentrations range from 2.3 to 25.0 μmol mol^{-1} creatinine as equal to the US TLV of 0.2 mg m^{-3} as BSM or equal to the German TRK of 2 μg m^{-3} benzo(*a*)pyrene. It might be that the higher dermal exposure level at some cokeovens is responsible for the higher level of urinary 1-hydroxypyrene at the OEL concentration. The end-of-shift concentration of 1-hydroxypyrene of 2.3 μmol mol^{-1} creatinine is proposed as the third level of the benchmark guideline, valid solely for cokeovens.

In workers from the primary aluminium industry the lowest reported concentration of 1-hydroxypyrene in urine at the end of a shift, which is equal to the TLV of 0.2 mg m^{-3} as BSM, is 4.9 μmol mol^{-1} creatinine. The relative proportion of pyrene in the PAH mixture of different worksites (paste plant, bakeoven and potrooms) in primary aluminium production varies widely. This may be the reason for the highly deviating levels of urinary 1-hydroxypyrene at the OEL exposure level. The lowest reported concentration of 1-hydroxypyrene, namely 4.9 μmol mol^{-1} creatinine is proposed as the third level of the benchmark guideline, and is valid solely for primary aluminium production. The benchmark proposal for primary aluminium workers fits quite nicely with the proposal that was recently published by Bouchard and Viau (1999) based on ratios between pyrene and benzo(*a*)pyrene equivalents.

Knowing that the US TLV of coal tar pitch volatiles (CTPV) as benzene soluble matter (BSM) has not been based on clear health risk levels from epidemiological studies, but rather on analytical demands (detectable concentration of benzene soluble matter), it should be clear that the TLV of CTPV is not a limit value, solely based on health risks, but is more or less a practical compromise (ACGIH, 1998). Also the German TRK (Technische Richt Koncentration, that is Technical Guide Concentration) is based on the ALARA principle (as low as reasonably achievable). In The Netherlands, a health-based calculated OEL has recently been calculated for airborne benzo(*a*)pyrene using the linear extrapolation model to zero dose from tumour incidences at the risk level of 1:10 000 excess cancers per year for lifetime exposure—the estimate for the health-based calculated OEL was 0.315 μg m^{-3} benzo(*a*)pyrene (Netherlands Health Counsel, 1994). It is clear that this estimate is far below the existing US TLV or German TRK. One should therefore realize that the third level of the benchmark guideline of urinary 1-hydroxypyrene is above a certain excess risk level. This conclusion is supported by data from studies of genotoxic effects in workers. These studies show lowest genotoxic effect levels in cokeoven workers at a concentration of 1-hydroxypyrene of 1.9 μmol mol^{-1} creatinine (increased level of single-strand breaks) and in the primary aluminium workers at 3.8 μmol mol^{-1} creatinine (increased level of PAH–DNA adducts). A complicating fact is that the results of the studies of PAH–DNA adducts in aluminium and electrode paste plant workers are not consistent (Cartensen *et al*., 1999). Among four studies, only one study reported a dose-related increase in DNA adducts (van Schooten *et al*., 1995).

In a number of studies the level of 1-hydroxypyrene in workers who smoke and are exposed to PAH is higher than the level in non-smoking colleagues; this indicated the need for a separate first level of the benchmark guideline for smokers and non-smokers. The second and third levels of the benchmark guideline, however, cannot be set for smokers and non-smokers separately, since the majority of the present available studies of biological monitoring of exposure and monitoring of genotoxic effects among PAH-exposed workers are not reported for these two groups, split by smoking habits.

No other international governmental organization has proposed a biological exposure limit yet. Lauwerys (1997) proposed in the chapter 'Occupational Toxicology' of the 5th edition of Cassett and Doull's *Toxicology* a tentative urinary 1-hydroxypyrene limit value of 2.7 μg g^{-1} (equal to 1.4 μmol mol^{-1}).

Several enzymes like the cytochrome P-450 1A1 and the glutathion transferases may mediate the metabolism of pyrene. The capacity of cytochrome P-450 1A1 may vary between individuals and inter-individual differences exist. The meaning of the genetic polymorphism of cytochrome P450 1A1 and glutathione transferases to modulate the relationship of individual exposure to pyrene to individual urinary 1-hydroxypyrene concentrations is still too limited to introduce this as a clear modulating factor (Ovrebo *et al*., 1998; Wu *et al*., 1998b; Pan *et al*., 1998).

Only when results from epidemiological studies of workers with long-term exposure to PAH, with urinary 1-hydroxypyrene as a dose indicator, will a more reliable estimate of the average 1-hydroxypyrene level versus excess tumour incidence be made. Only with these data will an accurate and precise estimate of the health-based limit value of urinary 1-hydroxypyrene be made. A guideline based on epidemiological data for exposed workers will be superior to the three-level benchmark guideline presented here and such a standard will supersede the curent proposal when such studies are available.

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