Polycyclic Aromatic Hydrocarbon Metabolites in Urine as Biomarkers of Exposure and Effect

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Humans are exposed to polycyclic aromatic hydrocarbons (PAHs) from various occupational, environmental, medicinal, and dietary sources. PAH metabolites in human urine can be used as biomarkers of internal dose to assess recent exposure to PAHs. PAH metabolites that have been detected in human urine include 1-hydroxypyrene (1-OHP), 1-hydroxypyrene-*O*-glucuronide (1-OHP-gluc), 3-hydroxybenzo[*a*]pyrene, 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, and a number of other hydroxylated PAHs. The most widely used of these is 1-OHP-gluc, the major form of 1-OHP in human urine, by virtue of its relatively high concentration and prevalence in urine and its ease of measurement. This metabolite of pyrene can be measured as 1-OHP after deconjugation of the glucuronide with β-glucuronidase or directly as 1-OHP-gluc without deconjugation. Elevated levels of 1-OHP or 1-OHP-gluc have been demonstrated in smokers (versus nonsmokers), in patients receiving coal tar treatment (versus pretreatment), after workshifts in road pavers (versus before shifts or versus controls), after shifts in coke oven workers (versus before shift), and in subjects ingesting charbroiled meat (versus preingestion). More importantly, this metabolite is found (at low levels) in most human urine, even in persons without apparent occupational or smoking exposure. Although measurement of these metabolites is useful in assessing recent exposure to PAHs, their value as predictive markers of biological effect or health outcomes has not been rigorously tested and at present can only be inferred by association. — Environ Health Perspect 104(Suppl 5):927–932 (1996)

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced during incomplete combustion of organic materials, and some are known to be carcinogenic or cocarcinogenic. Humans may be exposed to these compounds from a wide variety of sources, including occupation (coke oven or iron foundry workers), environment (air pollutants, drinking water), personal habits (e.g., smoking), medical treatment (coal tar), and diet (broiled and smoked foods) (*1–8*). PAHs are readily absorbed into the body through the skin, lungs, and gastrointestinal tract. Exposure to PAHs has been associated with lung and skin cancer in occupational settings and may be associated with other human cancers (*9–13*). In addition to their carcinogenic properties, PAHs are known to induce metabolic enzymes and are cytotoxic.

The measurement of PAH metabolites in urine has been proposed as a means of assessing recent exposure to these compounds. Besides the general advantages of urinary markers such as noninvasiveness and ready accessibility, urinary PAH metabolites are useful when assessing multiple routes of exposure, when assessing external exposure is difficult, or when collecting blood is unacceptable. The measurement of carcinogen metabolites in urine has several potential uses: *a*) identification of groups and/or individuals with recent exposure and, as a result, possibly elevated risk of adverse health effects; *b*) improvement of exposure information in general and examination of geographic or household differences; *c*) improvement of risk extrapolation between species; and *d*) understanding the etiology of and susceptibility to human carcinogenesis. For urinary PAH biomarkers to be validated for wide application in human populations, a number of relevant factors should be examined: inter- and intraindividual variation, sensitivity and specificity of the measuring methods, and knowledge about the kinetics of occurrence and persistence of the biomarker (*14*).

Urinary PAH Metabolites as Biomarkers of Internal Dose

PAH Metabolites Detected

A number of urinary markers of exposure to PAHs have been investigated to assess recent exposure to these compounds. Becher and Bjørseth (*15*) developed an analytical procedure to measure PAHs in human urine by chemically reducing the metabolites to parent PAHs and subsequent analysis by HPLC with fluorescence detection. Although this approach increases the sensitivity of the assay by increasing the amount of parent compound used in the analysis, a considerable body of information about individual metabolites (and their ratios) is lost in this process. The recovery of the assay varied between 10 and 85% depending on the individual compound, and the amount of compound detected increased about 5-fold after reduction of metabolites. Using this methodology, 11 urinary PAHs were quantitated in aluminum plant workers and controls stratified by smoking status (*16*). Total PAH levels were elevated in occupationally exposed workers and current smokers compared to nonsmoking controls; however, when some individual PAHs, including benzo[*a*]pyrene (B[*a*]P), were examined, no differences were observed. In addition, the assay was not sensitive enough to examine the relationship between the air concentration of PAHs and the excretion

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Abbreviations used: 1-OHP, 1-hydroxypyrene; 3-OH-B[*a*]P, 3-hydroxybenzo[*a*]pyrene; B[*a*]P, benzo[*a*]pyrene; ELISA, enzyme-linked immunosorbent; 7,8-diol-B[*a*]P, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene; B[*a*]P-tetro, l7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; IAC, immunoaffinity chromatography; PAHs, polycyclic aromatic hydrocarbons; SFS, synchronous fluorescence spectroscopy.

of PAHs in human urine. Further application of this method (reversed metabolism) to urine samples from workers in aluminum plants in Italy (*17*) and from coke oven workers (*18*) did not show differences in PAH levels in urine between exposed workers and nonexposed controls.

Keimig et al. (*19*) reported that 1-hydroxypyrene (1-OHP) is a major metabolite of pyrene in pig urine using high performance liquid chromatography (HPLC) with fluorescence detection and gas chromatography–mass spectrometry (GC–MS) confirmation. Jongeneelen et al. (*20–24*) subsequently performed a series of studies evaluating the excretion of PAH metabolites in rodent and human urine. They also measured 3-hydroxybenzo-[*a*]pyrene (3-OH-B[*a*]P) in rat urine by HPLC analysis after oral administration of B[*a*]P given in different dose levels (*25*). Because the urinary concentration of 3-OH-B[*a*]P was 2500-fold lower than that of 1-OHP in psoriatic patients undergoing dermal coal tar treatment, these investigators suggested that measurement of 1-OHP in urine by HPLC would be a more sensitive assay to monitor occupational exposure to PAHs (*22*). A number of studies were conducted to measure 1-OHP in urine of individuals exposed to PAHs occupationally and therapeutically. These included coke oven workers, petroleum coke handlers, operators of a creosote-impregnating plant, psoriatic patients treated with mineral coal tar, and eczematous dermatitis patients undergoing coal tar ointment treatment. Although some specific PAH metabolites were identified in urine after exposure to PAHs, it was impossible to characterize all of the isolated metabolites. Some of these might be more important than 1-OHP as biomarkers of exposure to PAHs in terms of health effects and biological relevance (*20,23,24,26*).

In a recent study (*27*), pyrene metabolites were measured in human urine before enzymatic deconjugation. The analytical method used immunoaffinity chromatography (IAC) using anti-PAH adduct antibody, HPLC with fluorescence detection, and the complementary techniques of synchronous fluorescence spectroscopy (SFS) and GC–MS to measure pyrene-containing metabolites. The majority of urinary 1-hydroxypyrene was conjugated to glucuronide. Because 1-hydroxypyrene-glucuronide is approximately 5-fold more fluorescent than 1-OHP, it provides a more sensitive biomarker for assessing exposure to pyrene.

Gomes and Santella (*28*) investigated methods for the detection of B[*a*]P metabolites in rodent urine by applying several different preparative methods (ethyl acetate extraction, Sep-pak C18 cartridge chromatography, XAD-2 resin chromatography, and IAC using anti-PAH adduct antibody) and quantitation of metabolites by competitive enzyme-linked immunosorbent assay (ELISA). They concluded that metabolites are isolated best (52% recovery) by IAC. Because the monoclonal antibodies used to prepare their immunoaffinity columns and used in their ELISAs showed cross-reactivity with other B[*a*]P and PAH metabolites, they suggested that this assay may be useful as a general indicator of exposure to PAHs.

More recently, Weston et al. (*29*) reported an assay for quantitation of B[*a*]P-tetrol in human urine by sequential use of several analytical methods, IAC, HPLC, and SFS. Although the sensitivity of the assay was good (limit of detection was 0.01 pmol/ml urine for a 10-ml urine sample), the overall recovery of the assay (about 30%) could be improved to enhance the sensitivity of the assay. In spite of the low recovery rate of the assay, 0.24 to 3.12 pmol B[*a*]P-tetrol/ml urine was detected in four individuals who had recently consumed charbroiled beef.

Further application of HPLC and GC–MS methodologies [(*30*); S Myers, unpublished data] have identified additional hydroxylated and methylated PAHs in human urine. Of particular interest are the methylated PAH metabolites, which have not been studied in great detail but are highly mutagenic.

Association of PAH Metabolite Levels with PAH Exposure in Human Studies

Levels of 1-OHP in urine from individuals exposed to several different PAH sources are summarized in Table 1. Elevated levels of 1-OHP have been demonstrated in smokers, in patients after coal tar treatment, after workshifts in road pavers, after shifts in coke oven workers, after shifts in aluminum reduction workers, and in subjects ingesting charbroiled meat (*20–24,31–35*). These results indicate that considerable variability occurs in these measurements when performed by different investigators at different worksites. Identifying the relative contribution of several potential sources of variability (e.g., laboratory, exposure, or biological differences) is the focus of much of the current research in this area.

Kinetics and Interindividual Variation in Metabolite Excretion

An understanding of the excretion kinetics of PAH metabolites is necessary for their effective use as indicators of exposure. Studies in occupationally exposed workers suggest that the half-life for urinary excretion of 1-OHP is about 18 hr (*36*) and ranges from 6 to 35 hr (*24*). Significant differences in preshift and postshift concentrations of urinary 1-OHP are consistent with overnight (16 hr) clearance of the internal dose of pyrene received on the previous day (*31*). The major routes of occupational exposure to pyrene in these groups are through inhalation and cutaneous absorption.

Additional information on excretion kinetics is obtained from PAH ingestion studies. Urinary excretion of PAH metabolites has been examined in several controlled feeding protocols (*34,35,37*). The kinetics of 1-OHP excretion was examined during and after ingestion of broiled beef containing PAHs (*34*). Ingestion of 250 g of broiled meat resulted in a 4- to 12-fold increase in urinary 1-OHP concentration. Maximum excretion occurred at approximately 6.3 hr after ingestion and declined thereafter with an estimated half-life of 4.4 hr. Thus, most of the 1-OHP would be excreted within about 15 hr (two half-lives after maximum). In another charbroiled beef feeding study (*35*), urinary 1-OHP was markedly elevated within 16 to 20 hr of ingestion and remained marginally elevated (2.5-fold above prefeed baseline) 72 hr after ingestion.

Biologically determined interindividual variation is difficult to assess in occupational exposure settings because of the uncertainty of true exposure and dose. However, controlled feeding studies allow a comparison of internal dose biomarkers with virtually identical exposures because food can be centrally prepared, homogenized, and weighed. Under such conditions (*35*), interindividual variation (8-fold) was observed among 10 subjects ingesting identical quantities of charbroiled beef. Thus, major individual differences in absorption, metabolism, and/or excretion of pyrene are indicated.

Use of PAH Metabolites in the Study of Biological Effect and Risk Assessment

Animal Studies

In animal models, the majority of pyrene is excreted in the urine and bile as glucuronide

Table 1. Levels of 1-hydroxypyrene in human urine.

Group	n	1-Hydroxypyrene, µmol/mol creatinine (range)	Reference
Nonsmokers	14	0.17 (0.01–0.93)	(24)
Smokers	28	0.51 (0.04–1.24)	
Asphalt pavers			(23)
Preshift	43	1.35	
Postshift	43	1.76	
Aluminum plant workers			(31)
Control	46	(0.44–0.61)	
Preshift	55	(0.43–0.77)	
Postshift	55	(1.93–3.60)	
Coke oven workers			(24)
Preshift	44	0.89 (0.24–3.50)	
Postshift	44	2.47 (0.46–11.2)	
Foundry workers			(32)
Nonsmokers			
Controls	20	0.16 (0.01–0.22)	
Exposed	16	0.11 (0.09–0.13)	
Smokers			
Controls	26	0.26 (0.18–0.34)	
Exposed	20	0.42 (0.25–0.59)	
Steel plant workers[a]			(33)
Nonsmokers			
Low PAH	20	0.05	
Mid-PAH	15	0.15	
High PAH	5	0.37 (0.02–0.89)	
Smokers			
Low PAH	20	0.06	
Mid-PAH	12	0.19	
High PAH	12	0.82 (0.15–3.57)	
Coal tar patients			(20,22)
Pretreatment	4	(1–20)	
Posttreatment	4	(100–500)	
PAH in diet			(34)
Preconsumption	5	0.09 (0.01–0.58)	
Postconsumption	5	1.51 (0.80–2.01)	
PAHs in diet[a]			(35)
Preconsumption	10	0.02 (0.01–0.14)	
Postconsumption	10	0.38 (0.08–1.10)	

PAHs, polycyclic aromatic hydrocarbons. [a]Measured as 1-hydroxypyrene-glucuronide.

or sulfate conjugates of hydroxylated pyrene metabolites (e.g., 1-OHP, 1,6-dihydrodiolpyrene, or 1,8-dihydrodiolpyrene) (*38*). The primary route of excretion of higher molecular weight PAHs (e.g., B[*a*]P) is via the feces (*10*), with only about 1 to 3% of B[*a*]P dose administered being recovered as urinary metabolites in rats (*39,40*) or 12% as urinary metabolites in rabbits (*41*).

Camus et al. (*42*) compared urinary B[*a*]P metabolite concentrations in two strains of mice with different susceptibility to PAH-induced carcinogenesis due to their different capacities to metabolize and activate PAHs. The more susceptible strain excreted more 3-OH-B[*a*]P than the resistant strain, consistent with the more rapid metabolism in the susceptible strain. However, less than 0.1% of the administered dose was detected as 3-OH-B[*a*]P in the urine. Similarly, Jongeneelen et al. (*43*) reported that cumulative excretion of 3-OH-B[*a*]P in rat urine was only 0.22 to 0.35% of administered dose after three consecutive high doses of B[*a*]P, or 0.11 to 2.3% of administered dose after one relatively low dose.

Further studies are required to assess the value of urinary PAH metabolite levels as potential indicators of risk as well as exposure. Likhachev et al. (*44*) suggested examining ratios of B[*a*]P metabolites in the activation versus deactivation pathways of B[*a*]P. For example, they monitored excretion of 7,8-diol-B[*a*]P and 3-OH-B[*a*]P in urine and feces of rats in an effort to predict individual tumor risk.

Association of PAH Metabolites with PAH–DNA Adducts in Humans

A limited number of studies in humans have examined the association between urinary biomarkers of PAH internal dose and biomarkers of macro-molecular dose (DNA adducts). In foundry workers, increasing levels of urinary 1-OHP ($r = 0.27$, $p = 0.07$ for trend) or peripheral blood PAH–DNA adducts ($r = 0.28$, $p = 0.08$) were observed with increasing external exposure (*45*). However, the correlation coefficient between the two biomarkers was only 0.15 ($p = 0.37$, $n = 38$). In contrast, the correlation between these two biomarkers in a study of aluminum plant workers was highly significant ($r = 0.66$, $p < 0.001$, $n = 23$) for smokers and marginally significant ($r = 0.25$, $p < 0.089$, $n = 31$) for nonsmokers (*46*). In a smaller study of dietary exposure to PAHs (*35*), these two biomarkers were also significantly correlated ($r = 0.79$, $p < 0.01$). The different results observed in these studies may be due to differences in the period of collection of urine samples in the studies.

Risk Assessment

Assessment of human disease risk associated with specific agents is based primarily on data from animal studies. Extrapolation of disease risk between species necessitates an assumption of comparable potency for a given dose. One approach toward addressing this assumption at the molecular level involves the use of target organ molecular damage (carcinogen–DNA adduct levels in the case of cancer) for predicting disease outcome (*47*). Although this methodology has been used to improve exposure assessment in epidemiological studies, more information is needed to judge its usefulness in predicting cancer risk across species

Biological monitoring in the workplace is useful for evaluating individual exposure; however, the dose–response relationship for adverse health effects due to that exposure is rarely known. Estimates of group disease risk associated with exposure can be approximated from urinary markers of internal dose and air measurements of external exposure in occupations with high disease prevalence. This approach was used by Jongeneelen (*48*) in an attempt to estimate a biological exposure limit value for urinary 1-OHP based on the airborne threshold limit value of coal tar pitch volatiles. In a study of PAH exposure in coke oven workers, measurements of urinary 1-OHP and PAH in personal air samples were used to construct an empirical

mathematical relationship between the two variables. Applying relative risk estimates of lung cancer for topside versus non-topside coke oven workers, it was possible to establish an indirect relationship between lung cancer mortality risk and urinary 1-OHP concentration (*48*) for coke oven workers. (It should be noted that this relationship would be expected to be different for other routes of exposure, other mixtures of PAHs, and other sources of PAHs.) Ultimately, validation of these results will require prospective studies of disease risk.

Conclusions

Relevance for Large-scale Population Monitoring

Biomonitoring is commonly performed using either urine or blood samples. The use of urine has advantages in that it is obtainable in large quantities, at multiple time points, and by self collection. In some circumstances, blood collection may not be medically or culturally acceptable. Depending on the assay to be performed, urine samples usually do not require processing before being frozen. In some cases, solid-phase extraction of biomarkers from urine before shipment and analysis may be convenient (*49*). In all cases, the stability of the metabolites of interest under planned storage and shipping conditions must be evaluated. A potential disadvantage of urine biomarkers is that, in general, they reflect only recent exposure (e.g., within 24–72 hr). However, multiple sampling can be used to address this issue.

Such biomarkers provide an alternative approach to improve assessment of exposure in experimental and epidemiologic studies. This approach has several potential applications: *a*) to supplement and refine exposure information obtained by questionnaire or records, particularly when multiple sources of exposure are present, *b*) in prospective population studies where biological samples are banked for later analysis, *c*) in case–control studies where exposure conditions are not affected by outcome, and *d*) in prevention studies where intermediate markers of dose or effect provide early indicators of change. These types of studies should ultimately lead to more accurate risk assessment and risk identification.

Need for Validation in Transitional and Prospective Studies

The use of biomarkers in epidemiologic studies to enhance exposure assessment, explore disease mechanism, and assess acquired and inherited susceptibility encompasses several objectives and study designs. These activities are often categorized as laboratory, transitional, etiologic, and public health applications (*50*). Optimal use of specific biomarkers (or classes of biomarkers) requires careful consideration of the objectives to be addressed and the appropriate study design. A useful discussion of this issue and a biomarker-study design matrix are presented by Rothman et al. (*50*). The advantages, disadvantages, and logistical characteristics of the proposed biomarker should be considered in planning the study.

Ongoing validation studies are assessing the usefulness of various biomarkers as indicators of carcinogen exposure, effect, and risk. One of the first rigorous tests of DNA adduct concentration as a predictor of human cancer risk was reported by Ross et al. (*51,52*). They examined the association between aflatoxin B_1–guanosine adduct level in urine and subsequent risk of liver cancer in a nested case–control design. Aflatoxin adducts and metabolites were assayed in previously collected and banked urine samples from liver cancer cases and matched controls. Detectable levels of the aflatoxin adducts and several other aflatoxin metabolites in urine were predictive of liver cancer development. In addition, evidence for a strong interactive effect between urinary aflatoxin biomarkers and hepatitis B virus was observed for liver cancer risk. This study demonstrates the potential value of using a biomarker of internal or molecular dose as a predictor of biological effect and health outcome. As biomarkers for other compounds are validated, they will enhance the options available to epidemiologists in designing studies to understand and prevent human disease.

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