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The determination of polycyclic aromatic hydrocarbons in the urine of non-smoking Polish pregnant women

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HIGHLIGHTS

- 2-, 3-, 9-OH-PHE and PHE-9,10-diol are sufficient for PAH exposure assessment.
- Residential heating, living in a city center and ETS are predictors of PAH exposure.
- Women in Poland suffer from higher PAH exposure than in other western countries.

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ABSTRACT

The aim of this study was to characterize the PAH exposure level among the non-smoking Polish pregnant women and to identify the minimal set of PAH metabolites that specifically reflect environmental PAH exposure. The study population consisted of 210 non-smoking pregnant women. The urine sample was used for analysis of the following PAH metabolites: 1-, 2-, 3-, 4-, 9-hydroxyphenanthrene (1-, 2-, 3-, 4-, 9-OH-PHE), 1-hydroxypyrene (1-OH-PYR), 1,6 + 1,8-dihydroxypyrene (DI-OH-PYR), phenanthrene *trans*-1,2-dihydrodiol (PHE-1,2-diol) and phenanthrene *trans*-9,10-dihydrodiol (PHE-9,10-diol). The analysis of all the biomarkers was performed by gas chromatography–mass spectrometry after their derivatization. The mean PAH metabolite concentrations were in the range of 0.15 (±0.2) μg/g creatinine for 9-OH-PHE to 5.9 (±10.6) μg/g creatinine for PHE-9,10-diol. Women living in the city center had higher concentrations of 1-OH-PHE ($\beta = 0.6$; $p = 0.04$), 3-OH-PHE ($\beta = 0.8$; $p = 0.02$), 9-OH-PHE ($\beta = 0.9$; $p = 0.02$), and DI-OH-PYR ($\beta = 1.0$; $p = 0.006$) than those living outside the city center. The usage of coal for residential heating was a significant predictor of all PAH metabolites except for 9-OH-PHE ($p = 0.1$) and PHE-9,10-diol ($p = 0.08$). With the increasing cotinine levels we observed a significant increase in the concentrations of the following PAH metabolites: 3-OH-PHE ($\beta = 0.2$; $p = 0.007$), 4-OH-PHE ($\beta = 0.3$; $p = 0.002$), PHE-1,2-diol ($\beta = 0.3$; $p < 0.001$), 1-OH-PYR ($\beta = 0.2$; $p = 0.01$). High-density housing, usage of coal for residential heating, cotinine level in saliva, season of urine collection and distance from the place of residence to the main road explained 26% of the variance of 3-OH-PHE and 21% of the variance of 1-OH-PHE. 2-OH-PHE, 3-OH-PHE, 9-OH-PHE and PHE-9,10-diol are sufficient to predict environmental PAH exposure. The urinary PAH biomarker levels found in this study indicate that non-smoking Polish pregnant women suffer from a higher PAH exposure than those in other western countries. This higher PAH exposure level probably poses a significant health risk for the newborns and young children and will require further attention in the future.

Abbreviations: PAH, polycyclic aromatic hydrocarbons; 1-, 2-, 3-, 4-, 9-OH-PHE, 1-, 2-, 3-, 4-, 9-Hydroxyphenanthrene; Σ OH-PHE, sum of 1-, 2-, 3-, 4-, and 9-OH-PHE; PHE-1,2-diol, phenanthrene *trans*-1,2-dihydrodiol; PHE-9,10-diol, phenanthrene *trans*-9,10-dihydrodiol; Σ PHE-diol, sum of PHE-1,2-diol and PHE-9,10-diol; 1-OH-PYR, 1-Hydroxypyrene; DI-OH-PYR, sum of 1,6- and 1,8-dihydroxypyrenes; Σ OH-PYR, sum of 1-OH-PYR and DI-OH-PYR.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are a well-known class of environmental pollutants (Grimmer, 1983) that usually occur as complex mixtures of more than 300 compounds composed of fused aromatic rings. They are released into the ambient air during incomplete combustion and pyrolysis of organic material such as fuel, coal, wood, garbage, and tobacco. The corresponding PAH sources include emissions from motor vehicles and trucks, coal-fired power plants, residential heating, and tobacco smoking. Furthermore, food provides another important source of PAH which are formed predominantly as undesired contaminants during industrial food processing e.g. heating, drying and smoking, as well as domestic cooking procedures such as grilling and roasting (Phillips, 1999; EFSA, 2008).

Several PAH have been classified by the International Agency for Research on Cancer (IARC) as human carcinogens or probably or possibly carcinogenic to humans (IARC, 2010). However, growing evidence supports the association between prenatal exposure to PAH and intrauterine growth restriction, small-for-gestational age and preterm delivery (Perera et al., 2005; Choi et al., 2006, 2008a, 2012; Tang et al., 2006; Jedrychowski et al., 2012). In addition, recent data indicate that PAH exposure can result in impaired child neurodevelopment such as decreased cognitive and motor functions, reduced IQ and the increased risk of behavioral problems (Perera et al., 2009; Tang et al., 2008; Edwards et al., 2010; Wang et al., 2010; Perera et al., 2011).

After their inhalation, oral and/or transdermal uptake PAH undergo an oxidative metabolism involving cytochrome P450 (CYP450) enzymes and microsomal epoxide hydrolase (EPX1). Phenols and *trans*-dihydrodiols are derived from the initially formed arene oxides by a non-enzymatic rearrangement and a hydrolysis catalyzed by EPX1, respectively. Some *trans*-dihydrodiols can be further oxidized to reactive dihydrodiol epoxides which are subsequently excreted in part as tetrols (Conney, 1982; Thakker et al., 1985; Jacob, 2008). In particular, the metabolites of lower molecular weight PAH such as naphthalene, phenanthrene and pyrene are excreted in urine in substantial amounts, whereas metabolites of larger PAH such as benzo[*a*]pyrene are predominantly excreted via bile and occur in urine only in tiny amounts which are difficult to determine (Jacob and Seidel, 2002; Hecht et al., 2003; Nilsson et al., 2013). The major proportion of hydroxylated PAH metabolites are excreted in the urine as water-soluble conjugates such as glucuronides and sulfate esters (Jacob and Seidel, 2002).

The measurement of urinary PAH metabolites provides a valuable tool to assess the individual level of internal PAH exposure (Strickland et al., 1996; Jacob and Seidel, 2002; Al-Saleh et al., 2013). The most widely used urinary biomarkers for measuring PAH exposure are the phenolic metabolites formed from naphthalene (1- and 2-naphthol), pyrene (1-hydroxypyrene [1-OH-PYR] and the sum of 1,6- and 1,8-dihydroxypyrenes [Di-OH-PYR]) (Seidel et al., 2008) and phenanthrene (the 5 isomers: 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene [OH-PHE]) (Onyemauwa et al., 2009). Phenanthrene tetrol (PHE-T) has also been determined (Hecht et al., 2003; Hochalter et al., 2011; Nilsson et al., 2013). It is considered as a urinary surrogate marker for tetrols formed from carcinogenic PAH such as benzo[*a*]pyrene. Due to its structural similarity it reflects the metabolism of benzo[*a*]pyrene to its carcinogenic dihydrodiol epoxide (Hecht et al., 2003).

The published studies on evaluation of PAH exposure among pregnant women are mostly based on analyses of airborne PAH by personal air sampling or measurements of PAH-DNA adduct levels. Most of the so far performed studies in this field derive from the US and Polish cohorts of pregnant women. Such analyses indicate that the sources as well as proportions of specific compounds in the total PAH mixture differ widely across different locations.

Our previous analysis from the Polish Mother and Child Cohort indicated the following predictors of urinary 1-hydroxypyrene (1-OH-PYR) level: summer season of urine collection (1-OH-PYR geometric mean for May–August: 0.5 μg/g creatinine and for September–April: 0.3 μg/g creatinine; $p = 0.01$), smoking status (1-OH-PYR geometric mean for smokers: 0.7 μg/g creatinine and for non-smokers: 0.3 μg/g creatinine; $p < 0.001$) and living in big cities (1-OH-PYR geometric mean for the place of residence with >500 thousands inhabitants: 0.5 μg/g creatinine and for ≤500 thousand inhabitants: 0.3 μg/g creatinine; $p = 0.001$) (Polańska et al., 2011).

The aim of this study was to characterize the PAH exposure level among the non-smoking Polish pregnant women based on a variety of different urinary metabolites used as biomarkers and to identify the minimal separating set of PAH metabolites that specifically reflect environmental PAH exposure.

2. Materials and methods

2.1. Study design and population

The subjects included in this analysis constitute a part of the prospective Polish Mother and Child Cohort Study (REPRO_PL cohort) (Polańska et al., 2009; Supplementary material S1). The inclusion criteria were as follows: single pregnancy (up to 12 weeks of gestation), not assisted with reproductive technology and no serious chronic diseases specified in the study protocol.

The current analysis was restricted to 210 pregnant women from Lodz and Legnica districts who were non-smokers and had no history of occupational PAH exposure. The smoking status of the pregnant women was verified by determination of cotinine levels in saliva (collected between 20th and 24th weeks of gestation) using a stable isotope dilution LC–ESI-MS/MS method (high performance liquid chromatography coupled with tandem mass spectrometry/positive electrospray ionization) (Stragierowicz et al., 2013). Taking into account organizational reasons saliva not urine samples were selected for assessment of smoking status. However our previous analyses based on subsample from REPRO_PL cohort demonstrated a strong correlation of cotinine concentrations in saliva and urine in the second trimester of pregnancy ($r = 0.9$; $p < 0.001$) (Stragierowicz et al., 2013). All the women with the cotinine levels above 10 ng/ml were considered as smokers and were excluded from the analysis. The cut off point for the cotinine level was selected based on recommendations made by SRNT Subcommittee on Biochemical Verification (2002).

The current analysis was focused on the following metabolites of PAH exposure: 1-, 2-, 3-, 4-, 9-hydroxyphenanthrene (1-, 2-, 3-, 4-, 9-OH-PHE), 1-hydroxypyrene (1-OH-PYR) and 1,6 + 1,8-dihydroxypyrenes (DI-OH-PYR), phenanthrene *trans*-1,2-dihydrodiol (PHE-1,2-diol) and phenanthrene *trans*-9,10-dihydrodiol (PHE-9,10-diol). In our previous study (Polańska et al., 2011) 1-OH-PYR was determined by HPLC-FD. In this study 1-OH-PYR was determined again with a stabile-isotope dilution GC–MS method as part of the phenolic PAH profile analysis to get a consistent set of analytical data.

2.2. Questionnaires

All the women participating in the study were interviewed 3 times during pregnancy (once in each trimester) by a gynecologist or a midwife. The questionnaires were administered to collect data on demographic characteristics and socio-economic status (SES), medical history, previous and current pregnancies and environmental exposures during pregnancy. In order to explore the factors that may be predictive of PAH exposure, the current analysis was focused on variables related to the demographic and socio-economic characteristics (age, marital status, employment, education), dietary intake of PAH (frequency of grilled or fried food intake based on the food frequency questionnaire), home characteristics (type of cooking and heating method, distance of the place of residence from the main road based on the address), season of urine collection and passive smoking (based on the cotinine level in saliva). House density in the place