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Evaluation of 11 polycyclic aromatic hydrocarbon metabolites in urine of Czech mothers and newborns

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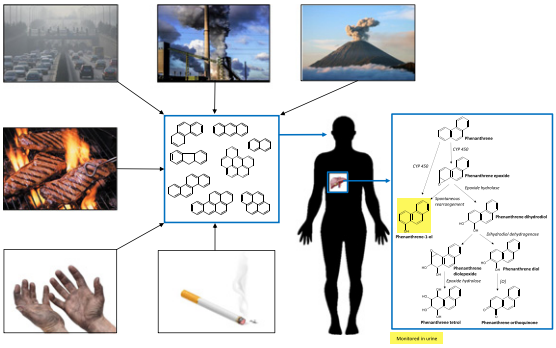
HIGHLIGHTS

- 11 OH-PAHs were measured in 531 urine samples from mothers and their newborns.
- The most frequently detected analyte with the highest concentration was 2-OH-NAP.
- Chrysene-6-ol and benzo[*a*]pyrene-3-ol were not detected in any of analyzed samples.
- ΣOH-PAHs in children's urine was1.6× lower compared to their mothers.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) represent a large group of ubiquitous contaminants of the environment, including food chain where they are released as by-products of incomplete combustion of an organic matter. Epidemiological studies have shown that exposure to PAHs correlated with increased incidence of cancer. Carcinogenicity is associated mainly with metabolites that are formed during metabolic degradation of these substances in exposed organism. In this study monohydroxylated PAHs (OH-PAHs), the major metabolites excreted into urine, were determined in 531 urine samples collected from mothers and their newborns from two localities of the Czech Republic – heavily air polluted Karvina and control locality of Ceske Budejovice and in two sampling rounds – August–October 2013 (summer, less air polluted season) and January–April 2014 (winter, more air polluted season). From all targeted analytes, naphthalene-2-ol was the most abundant compound present in 100% of the samples and it represented also the analyte with the highest concentration. Median concentration of ΣOH-

Abbreviations: 1-OH-NAP, naphthalene-1-ol; 1-OH-PHEN, phenanthrene-1-ol; 1-OH-PYR, pyrene-1-ol; 2-OH-FLUO, fluorene-2-ol; 2-OH-NAP, naphthalene-2-ol; 2-OH-PHEN, phenanthrene-2-ol; 3-OH-BaP, benzo[*a*]pyrene-3-ol; 3-OH-PHEN, phenanthrene-3-ol; 4-OH-PHEN, phenanthrene-4-ol; 6-OH-CHRY, chrysen-6-ol; 7-OH-BaP, benzo[*a*]pyrene-7-ol; 9-OH-PHEN, phenanthrene-9-ol; BaP, benzo[*a*]pyrene; BMI, body mass index; d-SPE, dispersive solid phase extraction; d_7-1-OH-NAP, $[^2H]_7$-naphtalene-1-ol; d_7-2-OH-NAP, $[^2H]_7$-naphtalene-2-ol; d_8-9-OH-PHEN, $[^2H]_8$-phenanthrene-9-ol; d_9-1-OH-PHEN, $[^2H]_9$-phenanthrene-1-ol; d_9-1-OH-PYR, $[^2H]_9$-pyrene-1-ol; d_9-2-OH-FLUO, $[^2H]_9$-fluorene-2-ol; d_9-2-OH-PHEN, $[^2H]_9$-phenanthrene-2-ol; d_9-3-OH-PHEN, $[^2H]_9$-phenanthrene-3-ol; d_{11}-3-OH-BaP, $[^2H]_{11}$-benzo[*a*]pyrene-3-ol; EFSA, European Food Safety Authority; ESI-, electrospray ionization (negative mode); FLUO, fluorene; HiVol samplers, High-Volume air samplers; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LLE, liquid-liquid extraction; LOQ, limit of quantification; MRM, multiple reaction monitoring; NIST, National Institute of Standards and Technology; OH-PAHs, monohydroxylated PAHs; PAHs, polycyclic aromatic hydrocarbons; PHEN, phenanthrene; PFP, pentafluorophenyl; $PM_{2.5}$, particles smaller than 2.5 μm; PYR, pyrene; S/N, signal to noise ratio; SCF, Scientific Committee on Food; SRM, Standard Reference Material; U-HPLC–MS/MS, ultra-high performance liquid chromatography coupled with tandem mass spectrometry.

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Czech Republic
Biomonitoring
Human exposure
3-OH-BaP

PAHs in the urine of children was on average 1.6 times lower compared to the respective mother which correlates with higher intake of PAHs by mothers. ΣOH-PAHs concentrations determined in mothers' urine collected in the summer were comparable in both localities. No significant increase occurred in Ceske Budejovice in winter, while in samples from the Karvina region a statistically significant difference ($\alpha = 0.05$) in the amount of ΣOH-PAHs was observed. The median concentrations of ΣOH-PAHs in mothers' urine samples in the winter were 1.5 times higher than in the summer in the same locality. The amounts of ΣOH-PAHs in newborns' urine from Karvina in the winter season were 1.5 times higher than in the summer collected in the same locality and 3.3 times higher when compared with the less polluted locality of Ceske Budejovice.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent an important group of environmental contaminants that may be formed and released during combustion/pyrolysis of organic matter. In addition to a wide range of anthropogenic emission sources (e.g. heavy industry, exhaust fumes) and natural processes (e.g. volcanic activity, forest fires) some culinary practices or industrial processes (e.g. drying, grilling, roasting, frying and smoking) may also be associated with the formation of PAHs. With regards to these multiple sources the occurrence of PAHs in the environment is ubiquitous. They can be found in various compartments such air, soil, water, sediment and also in food supplies (EFSA, 2008). It is worth noting that these pollutants occur here as complex mixtures consisting of PAHs with a different number of aromatic rings (typically 2–6). The incidence of the individual compounds in these mixtures varies largely not only within different localities but also between various seasons depending on the intensity of emissions from heavy industry, traffic or heating frequency in the winter season (Li et al., 2010).

The most common exposure pathway of a human body to PAHs is through the digestion of contaminated foods, which accounts for over 70% of the total PAH exposure for non-smokers. Another important exposure route is the inhalation of airborne PAHs, in the case of smokers tobacco smoke is the major source (Ma and Harrad, 2015; Moustafa et al., 2015). For occupationally exposed individuals the transfer of these substances through the skin may also play an important role (e.g. workers with asphalt or coal, firefighters) (Campo et al., 2010; Oliveira et al., 2016).

Many studies have suggested that PAHs may exhibit a wide range of toxic effects (Alshaarawy et al., 2016; Wang et al., 2016). In the past decade PAHs were evaluated by the International Programme on Chemical Safety (IPCS), the Scientific Committee on Food (SCF) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It has been concluded that 15 PAHs, namely benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene and 5-methylchrysene show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals in vivo (Boström et al., 2002; IARC, 2010; Onyemauwa et al., 2009; Zhong et al., 2011).

The fate of PAHs in the exposed organism is fairly complex. Carcinogenic and mutagenic PAHs can undergo at least three major metabolic pathways, which form highly reactive electrophilic intermediates capable of binding to cellular macromolecules, in particular proteins and nucleic acids. Following absorption, a rapid biotransformation process starts with phase I metabolism in which these compounds are oxidized by the hepatic cytochrome P450 (CYP 450) monooxygenases to form reactive epoxide intermediates followed by their reduction or hydrolysis yielding hydroxylated derivatives (OH-PAHs). In phase II metabolism, the OH-PAHs are conjugated to glucuronic acid or sulphate to increase water solubility (Li et al., 2006). OH-PAH conjugates with 2–3 benzene rings (low-molecular-weight OH-PAHs) are mostly excreted via urine and those with 4 or more benzene rings are mainly excreted via bile and faeces (high-molecular-weight OH-PAHs) (Campo et al., 2008; Onyemauwa et al., 2009; Ramesh et al., 2004; Zhong et al., 2011).

PAH metabolism in the human body is likely to be influenced by many individual factors such as age, gender, body fat percentage, metabolism of xenobiotics, physical characteristics (body mass index - BMI) and lifestyle (smoking, alcohol consumption, physical activity, location of residence, etc.). These predispositions can lead to the formation of multiple metabolites including epoxides, diolepoxides, monohydroxylated and polyhydroxylated PAHs, dihydrodiols or ortho-quinones of these substances (Campo et al., 2008; Onyemauwa et al., 2009; Yanxin et al., 2011).

PAHs may also interfere with hormones and act as endocrine disruptors (Annamalai and Namasivayam, 2015). Some of these contaminants can behave like estrogens or their antagonists (antiestrogens) and thus disturb estrogen-regulated processes in the body. Moreover, phenanthrene (PHEN) or fluorene (FLUO) have antiandrogen activity (Jedrychowski et al., 2013). After they enter the body, due to their non-polar character PAHs are transported to all lipid-rich tissues. Consequently, repeated and prolonged exposure to PAHs may lead to certain accumulation of these compounds in fat cells, liver and kidney (Hu et al., 2008; Pleil et al., 2010). Recent studies also suggest that nowadays widespread childhood obesity might be associated with exposure of the developing organism to these substances (Jedrychowski et al., 2013; Li et al., 2015; Scinicariello and Buser, 2014).

Due to the above mentioned human health effects of PAHs, a monitoring of an occurrence of metabolites in biological matrices represents an important tool for the assessment of human exposure. Currently the most common biomarkers for the evaluation of overall PAHs exposure are OH-PAHs. Of these biomarkers the most often used is pyrene-1-ol (1-OH-PYR), since its parent compound pyrene (PYR) is one of the principal components in PAHs mixture contaminating air. Because of the molecular symmetry of PYR only one monohydroxylated metabolite (unlike other PAHs) is originated from this parent PAH, so the 1-OH-PYR concentration in urine, to which it is primarily excreted, is relatively high and thus easy to measure. Nevertheless, PYR is not carcinogenic (due to the absence of a 'bay region' in the PYR molecule electrophilic diolepoxides, that belong to the most potent chemical mutagens reported so far, not being formed). The monitoring of its metabolite for the purpose of risk assessment is only based on the assumption that its levels to some extent correlate with benzo[*a*]pyrene (BaP) and other carcinogenic species co-occurring commonly with PYR in PAHs mixtures (Barbeau et al., 2011). However, as noted above, the PAHs compositional pattern in the environment and consequently in organisms varies largely depending on many factors. To obtain more accurate data monitoring more than one OH-PAH marker is needed. The metabolites of BaP, the key representative of carcinogenic PAHs involved in some environmental monitoring studies (Grova et al., 2016; Hecht, 2002; Lutier et al., 2016) might obviously be considered as suitable exposure biomarkers for human studies. Unfortunately, the detection of the most often targeted BaP metabolites benzo[*a*]pyrene-3-ol (3-OH-BaP) and benzo[*a*]pyrene-7-ol (7-OH-BaP) in urine is rather difficult, since they occur here at ultra-trace levels. This is not only due the multiple metabolic pathways that the parent BaP may undergo (yielding various metabolites) but also because the above mentioned OH-PAHs