Comparison of polycyclic aromatic hydrocarbon metabolite concentrations in urine of mothers and their newborns

Katerina Urbancova [a], Darina Dvorakova [a], Tomas Gramblicka [a], Radim J. Sram [a,b], Jana Hajslova [a], Jana Pulkrabova [a,*]

[a] University of Chemistry and Technology, Faculty of Food and Biochemical Technology, Department of Food Analysis and Nutrition, Technicka 3, 166 28 Prague, Czech Republic
[b] Institute of Experimental Medicine Medicine CAS, Videnska 1083, 142 20 Prague 4, Czech Republic

HIGHLIGHTS

- The most frequently detected analyte with the highest concentration was 2-OH-NAP.
- 6-OH-CHRY and 3-OH-BaP were not detected in any of the analysed samples.
- ΣOH-PAHs in children's urine samples were 1.7× lower compared to their mothers.
- ΣOH-PAHs in urine did not correlate with PAHs measured in the ambient air.

GRAPHICAL ABSTRACT
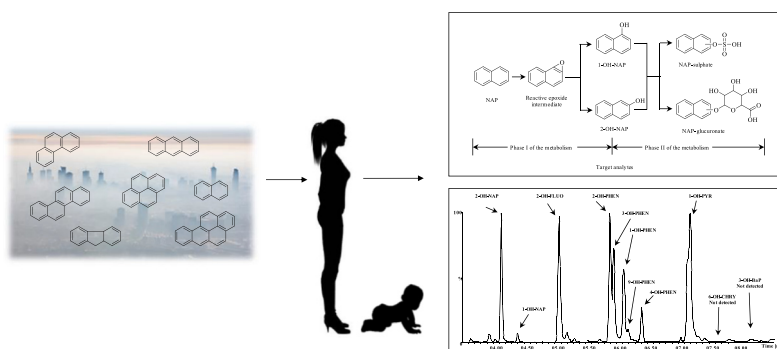
ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants produced during incomplete combustion of organic matter. Humans can be exposed to them via several pathways (inhalation, digestion, dermal exposure). The aim of this study was to assess the concentration of 11 monohydroxylated metabolites of PAHs (OH-PAHs) in 660 urine samples collected from mothers and their newborns residing in two localities of the Czech Republic — Most and Ceske Budejovice — in 2016 and 2017. After enzymatic hydrolysis, the target analytes were extracted from the urine samples using liquid-liquid extraction, with extraction solvent ethyl acetate and a clean-up step using dispersive solid-phase extraction (d-SPE) with the Z-Sep sorbent. For identification and quantification, ultra-high performance liquid chromatography coupled with tandem mass spectrometry was applied. 2-OH-NAP was the compound present in all of the measured samples and it was also the compound at the highest concentration in both mothers' and newborns' urine samples (median concentration 5.15 μg/g creatinine and 3.58 μg/g creatinine). The total concentrations of OH-PAHs in urine samples collected from mothers were 2 times higher compared to their children. The most contaminated samples were collected in Most in the period October 2016–March 2017 from both mothers (12.59 μg/g creatinine) and their newborns (8.29 μg/g creatinine). The concentrations of OH-PAHs in urine samples, which were collected from both mothers and their newborns as presented in this study, are comparable with those found in our previous study between 2013 and 2014. In addition, they are slightly lower or comparable to other studies from Poland, USA, Germany, China, and Australia.

* Corresponding author.
E-mail address: jana.pulkrabova@vscht.cz (J. Pulkrabova).

The results might indicate that the population in the previously highly air-polluted mining districts carries some long-term changes (maybe existing changes in genetic information), which also affect the metabolism of PAHs. It could be related to the long-lasting effect, and thus corresponding to the shortened life expectancy.

1. Introduction

PAHs are a group of well-known ubiquitous environmental contaminants that are formed during incomplete combustion or pyrolysis of organic matter (Kim et al., 2013). The most thoroughly investigated substance from this group is benzo[*a*]pyrene (BaP), which is listed as a carcinogen in humans (Group 1) according to a database by the International Agency for Research on Cancer (IARC). Another compound of high concern is chrysene (CHRY), which is listed as possibly carcinogenic to humans (Group 2B) (Li et al., 2016; IARC, 2018). However, PAHs occur in the environment as complex mixtures and thus it is necessary to monitor more than one compound (Yamamoto et al., 2015).

Humans can be exposed to PAH mixtures via various pathways, such as inhalation of contaminated air and/or tobacco smoke, or digestion of contaminated food. For occupationally exposed individuals (e.g., steel, coal or asphalt workers), dermal absorption is the major exposure pathway (Ma and Harrad, 2015; Alicandro et al., 2016). The high molecular PAHs (e.g., BaP or indeno[1,2,3-*cd*]pyren) tend to accumulate to some extent in fat tissue and only a minor part of them is metabolised. The low molecular PAHs (e.g. fluorene or phenanthrene) are metabolised in a larger part. After entering the body, PAHs are metabolised by cytochrome P450 enzymes. Metabolism includes two main phases: In Phase I, OH-PAHs are mainly formed. In Phase II, these compounds are conjugated with glucuronic acid or sulfate to produce compounds that are more water-soluble and are easily excreted from the body via urine or bile (Tombolini et al., 2018).

During their metabolic transformation, PAHs can become harmful to human health. In the first phase of metabolism, reactive species could be formed and interact with proteins or DNA and, as a result, exposure to PAHs can cause a higher incidence of cancer. PAH metabolites (mainly OH-PAHs) can act as endocrine disruptors, and therefore, can negatively affect the endocrine system. Exposure to PAHs during pregnancy can affect foetal development and can lead to intrauterine growth retardation and lower birth weights (Dejmek et al., 2000; Abdel-Shafy and Mansour, 2016). Prenatal PAH exposure can also be associated with the development of attention deficit hyperactivity disorder (ADHD) (Perera et al., 2018). Children from regions with higher PAHs exposure suffer from higher respiratory morbidity (Hertz-Picciotto et al., 2007; Dostal et al., 2013) and increased incidence of asthma bronchiale (Choi et al., 2017; Choi et al., 2019). Scientific research also indicates that PAHs (their metabolites) can affect childhood obesity (Poursafa et al., 2018). The International Programme on Chemical Safety (IPCS), the Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that 15 PAHs show clear evidence of mutagenicity and genotoxicity in somatic cells in experimental animals in vivo (EFSA (European Food Safety Authority), 2008). However, there are no regulations for the restriction of the levels of PAH metabolites in the human body and biological matrices. The only restricted compounds by the legislation are parent PAHs in some foods, and the concentrations of BaP are regulated in the air.

To assess the exposure of the human body to PAHs, it is important to monitor the parent PAHs in the ambient air and food as well as their metabolites in biological samples. Therefore, for this reason, mainly OH-PAHs are measured in the urine.

According to previous researchers, it can be seen that they are more focused on the analysis of OH-PAHs in urine samples (i.e., to evaluate PAH exposure) collected from a broad range of population groups, for example, highly exposed people, such as steel (Onyemauwa et al., 2009) or asphalt workers (Campo et al., 2010), smokers (Ramsauer et al., 2011), or the general population living in contaminated areas (Guo et al., 2013a). The most recent scientific papers are more focused on the population where PAH exposure could be most harmful, such as young children, adolescents (Sochacka-Tatara et al., 2018; Dobraca et al., 2018; Kelishadi et al., 2018), and pregnant women (Nethery et al., 2012; Adetona et al., 2013).

The presented research addresses a specific gap identified for PAHs within the frame of the European HBM4EU project in understanding the relationship between exposure and health. The main aim was to evaluate the concentrations of 11 OH-PAHs in the urine samples collected from July 2016 to August 2017 from mothers and their newborns residing in two localities of the Czech Republic — Ceske Budejovice and Most. The district of Ceske Budejovice in Southern Bohemia was selected as a control locality, according to our previous studies (Sram et al., 2016; Veleminsky Jr. et al., 2016). The district of Most is one of the mining districts in Northern Bohemia, which was characterised by significant air pollution due to power plants and local heating emissions in the '70s and '80s, and use of brown coal with a high content of sulphur. The outcome of this load was seen in the shortening of life expectancy by two years in both males and females. This did not change in the period 1990–2017 (Sram et al., 1996). Our previous study analysed the effect of air pollution by PAHs in a highly polluted locality — the Karvina district in Northern Moravia and the control district of Ceske Budejovice (Urbancova et al., 2017).

2. Materials and methods

Certified standards of OH-PAHs, namely 1-OH-NAP and 2-OH-NAP (both 1000 μg/mL methanol) were purchased from Absolute Standards, Inc. (USA). 3-OH-PHEN, 9-OH-PHEN, 1-OH-PYR and 3-OH-BaP (all 10 μg/mL acetonitrile) were purchased from Neochema (Germany) and 6-OH-CHRY (100 μg/mL acetonitrile) was obtained from AccuStandard® (USA). 2-OH-FLUO, 1-OH-PHEN, 2-OH-PHEN, 4-OH-PHEN and isotopically labelled analogues, specifically $[^2H]_7$-naphtalene-1-ol (d$_7$-1-OH-NAP), $[^2H]_7$-naphtalene-2-ol (d$_7$-2-OH-NAP), $[^2H]_9$-fluorene-2-ol (d$_9$-2-OH-FLUO), $[^2H]_9$-phenanthrene-1-ol (d$_9$-1-OH-PHEN), $[^2H]_9$-phenanthrene-2-ol (d$_9$-2-OH-PHEN), $[^2H]_9$-phenanthrene-3-ol (d$_9$-3-OH-PHEN), $[^2H]_8$-phenanthrene-9-ol (d$_8$-9-OH-PHEN), $[^2H]_9$-pyrene-1-ol (d$_9$-1-OH-PYR), $[^2H]_{11}$-benzo[*a*]pyrene-3-ol (d$_{11}$-3-OH-BaP) were supplied by Toronto Research Chemicals Inc. (Canada) in a solid form. Creatinine was delivered by Sigma-Aldrich (USA). The purity of all standards and their isotopically labelled analogues was at least 98%.

Individual OH-PAHs delivered as solids were dissolved according to the manufacturers' recommendations. Mixtures of OH-PAHs and their isotopically labelled analogues (d$_x$-OH-PAHs) were prepared in methanol. All solutions were stored at −20 °C in the freezer.

The Standard Reference Material® (SRM) 3673 (Organic Contaminants in Non-Smokers´ Urine) used for the method validation experiments was supplied by the US National Institute of Standards and Technology (NIST, USA).

2.1. Chemicals, reagents and other materials

Ethyl acetate, picric acid, enzyme β-glucuronidase (type HP-2, glucuronidase activity ≥100,000 units/mL, sulfatase activity ≤7500 units/mL), sorbent Supel™ QuE Z-Sep and polypropylene centrifuge tube filters (nylon, pore size 0.22 μm) were supplied by Sigma Aldrich (USA). HPLC gradient methanol was delivered by Merck (Germany). Unsterile

polytetrafluoroethylene (5.0 μm, Ø 25 mm) filters were purchased from Rotilabo® (Germany). 96-well microtiter plates were obtained from the Gama Group (Czech Republic).

2.2. Sample collection

The sample collection was carried out within the grant of the Czech Academy of Sciences, Strategy AV21, Qualitas and EU Horizon 2020 HBM4EU. The samples were collected in two localities of the Czech Republic — Ceske Budejovice and Most, namely in the Ceske Budejovice Hospital, Department of Obstetrics and Department of Neonatology and in the Most Hospital, Department of Obstetrics and Department of Neonatology. All the mothers´ urine samples were collected before delivery and newborns´ urine samples were collected on the second day after delivery as spot samples. The study was approved by the Ethics Committee of both hospitals and the Institute of Experimental Medicine of the CAS in Prague. Each mother was required to sign a written consent. A total of 660 samples (330 samples from mothers and 330 samples from their newborns) were collected. The sampling was carried out from July 2016 to August 2017. The urine samples were stored in the freezer at −20 °C before analysis.

Each mother filled in a questionnaire regarding her age, body mass index (BMI), current residency, eating habits and smoking habits. According to the data, all the mothers claimed that they did not smoke during the third trimester of their pregnancy and, therefore, were evaluated as non-smokers. Gestation age, date of child's birth, delivery type, birth weight, and gender were obtained from the questionnaire filled in by medical personnel. The summary of the information is shown in Table 1.

Within the Qualitas project, air samples were also collected for the analysis of PAHs. Air was sampled using Hi-Vol filters, and it was collected every 3 days (10 samples/month). The sample volume was 1627 m^3/24 h in each location in seasons July–September 2016, October 2016–March 2017 and April–May 2017.

2.3. Description of methods

2.3.1. Measurement of urinary creatinine

To normalise the urine concentration/dilution in individual samples for data comparability, the creatinine values were used. The creatinine concentration was measured using a Jaffe's spectrophotometric method according to our previous study (Lankova et al., 2016).

2.3.2. Analysis of OH-PAHs in urine

2.3.2.1. Extraction. The sample preparation procedure based on LLE with the extraction solvent ethyl acetate and a clean-up step, using d-SPE with a *Z*-Sep sorbent, is described in detail in our previous paper (Urbancova et al., 2017).

2.3.2.2. Instrumental analysis. The UHPLC–MS/MS analysis of 11 urinary OH-PAHs was performed using an Acquity Ultra-Performance LC system coupled with a triple quadrupole mass spectrometer Xevo TQ-S (both from Waters, USA) with electrospray ionisation in a negative ion mode (ESI-). Analytes were separated on a PFP (pentafluorophenyl) Kinetex column, Phenomenex (USA) (100 mm × 2.1 mm × 1.7 μm).

Measurement conditions are described in more detail in our previously published paper (Lankova et al., 2016).

2.3.2.3. Quality assurance/quality control and validation. The validation of the analytical method for the analysis of 11 urinary OH-PAHs and the spectrophotometric method based on Jaffe's reaction for creatinine determination are described in detail in our previous study [30]. The method accuracy was controlled by using the SRM 3673. Limits of quantification (LOQs) were 0.01–0.025 ng/mL with recovery 77–113% and repeatability (RSD) 3–14%. For 6-OH-CHRY and 3-OH-BaP, which were not certified in the SRM 3673, the performance parameters were measured by the analysis of an artificially contaminated urine blank sample. The recovery for 6-OH-CHRY was 95% (RSD 13%, LOQ 0.01 ng/mL) and for 3-OH-BaP, it was 97% (RSD 16%, LOQ 0.9 ng/mL).

Background contamination by the target analytes was also monitored. A procedural blank sample (deionised water was used instead of urine) was prepared together with each batch of samples. The blank sample contained traces of 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN and 1-OH-PYR (concentrations below 0.02 ng/mL urine). The concentration of contamination in the blank sample was subtracted from all samples prepared on the same day as the procedural blank sample.

2.3.3. Analysis of PAHs in Hi-Vol filters

The extraction of PAHs from the Hi-Vol filters is described in our other paper (Polachova et al., 2020). Briefly, the target PAHs from the filters were extracted using the mixture hexane:dichloromethane (1:1, *v/v*) for 7 h in a Soxhlet apparatus. The primary extract was redissolved after evaporation in hexane and purified from interfering co-extracts by solid-phase extraction (SPE) on a silica column. After another evaporation, the final extract was dissolved in isooctane and analysed for the presence of 24 PAHs, using gas chromatography, combined with high-resolution mass spectrometry (time of flight analyser (TOF)) in electron ionisation mode (GC-HRTOFMS-EI). LOQs of this method ranged from 0.0006 ng/m^3 air to 0.0012 ng/m^3 air with a recovery of 64–118% and repeatability of 4–19%.

3. Results

3.1. Overall urinary concentrations of 11 OH-PAHs in urine samples

A total of 660 urine samples (330 samples collected from mothers, 330 from their newborns) were analysed to detect the presence of 11 OH-PAHs. Table 2 shows the results of OH-PAH concentrations in urine samples collected from mothers and their newborns. In the mothers' urine samples, 2-OH-NAP was the most abundant compound found in 100% of the samples, followed by 1-OH-PHEN (99% of samples), 2-OH-PHEN (99% of samples) and 2-OH-FLUO (99% of samples). Other compounds were present in >89% of the measured samples. In the case of urine samples collected from newborns, the most abundant compound was also 2-OH-NAP (100% of samples), followed by 2-OH-PHEN (88% of samples), 1-OH-PHEN (86% of the samples) and 9-OH-PHEN (82% of samples). The abundance of the other OH-PAHs was lower. Monohydroxylated metabolites of carcinogenic CHRY and BaP, namely 6-OH-CHRY and 3-OH-BaP, were not detected in any of the measured samples collected from mothers or their newborns. This

Table 1
General information about mothers and their newborns — median (min-max).

	Ceske Budejovice			Most		
	Jul-Sep 2016	Oct 2016-Mar 2017	Apr-Aug 2017	Jul-Sep 2016	Oct 2016-Mar 2017	Apr-Aug 2017
Mother's age (years)	32 (18–42)	32 (20–43)	32 (22–41)	30 (19–41)	29 (18–44)	30 (18–43)
BMI	23 (16–44)	22 (16–40)	23 (19–43)	24 (18–41)	22 (16–42)	23 (17–37)
Gestation age (weeks)	39 (36–40)	39 (36–42)	39 (37–40)	40 (35–42)	39 (37–42)	39 (36–40)
Birth weight (g)	3360 (2240–4070)	3445 (2440–4610)	3590 (2140–4340)	3280 (2700–4190)	3425 (1300–4530)	3410 (2090–4360)

Table 2
Urinary concentrations of 11 OH-PAHs measured in 660 samples collected from Czech mothers and their newborns (μg/g creatinine[a]).

Analyte	LOQ[b]	Mothers (n = 330)					Newborns (n = 330)				
		Mean	Median	Min	Max	% positive samples	Mean	Median	Min	Max	% positive samples
1-OH-NAP	0.025	0.77	0.40	0.03	19.66	98	0.24	0.13	0.03	13.31	67
2-OH-NAP	0.025	7.30	5.15	0.56	42.62	100	6.09	3.58	0.46	41.12	100
2-OH-FLUO	0.025	0.33	0.23	0.07	4.15	99	0.17	0.08	0.03	1.06	72
1-OH-PHEN	0.010	0.43	0.26	0.04	13.82	99	0.45	0.13	0.01	4.86	86
2-OH-PHEN	0.010	0.27	0.17	0.03	5.36	99	0.21	0.09	0.01	3.08	88
3-OH-PHEN	0.010	0.10	0.06	0.01	1.99	96	0.05	0.01	0.01	0.57	52
4-OH-PHEN	0.010	0.48	0.17	0.01	16.06	94	0.03	0.01	0.01	0.30	45
9-OH-PHEN	0.010	0.97	0.45	0.05	22.06	89	0.37	0.26	0.01	3.36	82
1-OH-PYR	0.025	0.18	0.12	0.03	2.14	91	0.06	0.01	0.02	0.94	36
6-OH-CHRY	0.010	–	–	<0.01	<0.01	0	–	–	<0.01	<0.01	0
3-OH-BaP	0.900	–	–	<0.900	<0.900	0	–	–	<0.900	<0.900	0
ΣOH-PAH	–	11.12	8.96	1.83	78.76	100	8.48	5.15	0.46	47.62	100

When target analyte was below LOQ for the mean and median calculation 1/2 LOQ value was used.
  [a] Mean, median, minimum and maximum concentration of creatinine was 1.1, 0.92, 0.30 and 2.9 mg/mL urine.
  [b] LOQ was calculated with the median concentration of creatinine (0.92 mg/mL).

result is in good agreement with other published papers (Onyemauwa et al., 2009; Veleminsky Jr. et al., 2016; Sykorova et al., 2015; Li et al., 2014), and it is probably caused by urine not being the major excretion route for these more lipophilic compounds.

As shown in Table 2, the concentrations of all monitored OH-PAHs (ΣOH-PAHs) was 1.7 times higher in the urine samples collected from mothers (median ΣOH-PAHs 8.96 μg/g creatinine) compared to their children (median ΣOH-PAHs 5.15 μg/g creatinine).

2-OH-NAP was the compound present in all of the measured samples and it was also the compound found at the highest concentration in both mothers' and newborns' urine samples (median concentration 5.15 μg/g creatinine and 3.58 μg/g creatinine). The domination of 2-OH-NAP among other measured OH-PAHs was also found in other similar studies (Tombolini et al., 2018; Sochacka-Tatara et al., 2018; Cathey et al., 2018) and our previous study (Urbancova et al., 2017). It can be assumed that inhalation may be the dominant source of exposure for low molecular PAHs, such as NAP (Guo et al., 2013b). However, this compound was not measured in the Hi-Vol filters analysed in this study due to its high volatility. The median concentrations of approximately one order of magnitude lower were measured for the compound with the second-highest concentration, 9-OH-PHEN (mothers – 0.45 μg/g creatinine, newborns – 0.26 μg/g creatinine). The concentrations of other measured PAHs were about one order of magnitude lower. In addition, their profiles varied in the urine samples compared between mothers and their newborns. No correlation was found in the concentrations of target compounds measured in the urine samples collected from mothers and their newborns. The highest correlation was found for 2-OH-NAP, with the coefficient of determination R^2 0.79 in the urine samples collected in Ceske Budejovice from April to August 2017. However, these coefficients for this compound were lower in urine samples collected in other seasons in Ceske Budejovice and the second sampling locality Most (Table S1 in the Supplementary data). In addition, no relationship was found between concentrations of OH-PAHs found in the urine and mothers' age, and BMI and newborns' birth weight.

3.2. Comparison of OH-PAH concentrations in urine samples collected from mothers and their newborns according to sampling season and locality

The samples were divided into three subgroups based on the sampling periods in relation to the concentrations of BaP in the air, namely from July to September 2016 (BaP concentration in the air <1 ng/m³), from October 2016 to March 2017 (BaP concentration in the air >1 ng/m³) and from April to August 2017 (BaP concentration in the air <1 ng/m³).

The concentration data of all PAHs measured in the air are summarised in Table S2 in the Supplementary Data.

To evaluate the differences between groups of samples, a statistical *t*-test was performed. Detailed results from this test (t-value, t_α and *p*-value) are summarised in Table S3 in the Supplementary Data.

3.2.1. Urine samples collected from mothers and their newborns

As was earlier mentioned, the median concentration of ΣOH-PAHs in all urine samples was approximately 1.7 times higher in the urine samples collected from mothers (median concentration 8.96 μg/g creatinine) compared to their children (median 5.15 μg/g creatinine) as shown in Fig. 1.

In the samples collected in the period July–September 2016 from mothers living in Ceske Budejovice, the median concentration of ∑OH-PAHs was statistically significantly higher (α = 0.05) compared to their newborns (8.88 μg/g creatinine and 3.86 μg/g creatinine). The same trend was observed in Ceske Budejovice in the urine samples collected in the periods of October 2016–March 2017 and April–August 2017. However, in the last two periods, we observed a smaller difference in ∑OH-PAHs concentrations in urine samples collected from mothers compared to their children than in the first sampling period. In October 2016–March 2017, the median concentration of ∑OH-PAHs in mothers' urine was 1.9 times higher compared to their children (6.76 μg/g creatinine and 3.65 μg/g creatinine). In April–August 2017, the median concentration of ∑OH-PAHs in mothers' urine was 1.7 times higher compared to their children (5.82 μg/g creatinine and 3.43 μg/g creatinine) as shown in Fig. 1.

Concerning the second sampling locality, Most, a statistically significant difference (α = 0.05) was found between the urine samples collected from mothers and their newborns in the sampling period of July–September 2016. The median concentration of ∑OH-PAHs in mothers' urine from this period was 2.3 times higher compared to their children (11.42 μg/g creatinine and 4.96 μg/g creatinine). In the second sampling season (October 2016–March 2017), the difference between the median concentration of ∑OH-PAHs in mothers' and newborns' urine samples was lower. The median concentration of ∑OH-PAHs in the urine samples collected from mothers was 1.5 times higher compared to their children (12.59 μg/g creatinine and 8.29 μg/g creatinine). No statistical difference (α = 0.05) was found in the concentrations of OH-PAHs in samples collected from mothers and their newborns in April–August 2017 in Most (8.65 μg/g creatinine and 8.04 μg/g creatinine) (Fig. 1).

3.2.2. Urine samples collected from mothers living in Ceske Budejovice and Most

The median concentration of ΣOH-PAHs in the urine samples collected from mothers living in Ceske Budejovice in the first sampling round (July–September 2016) was statistically significantly higher (α = 0.05) compared to the samples collected from mothers between

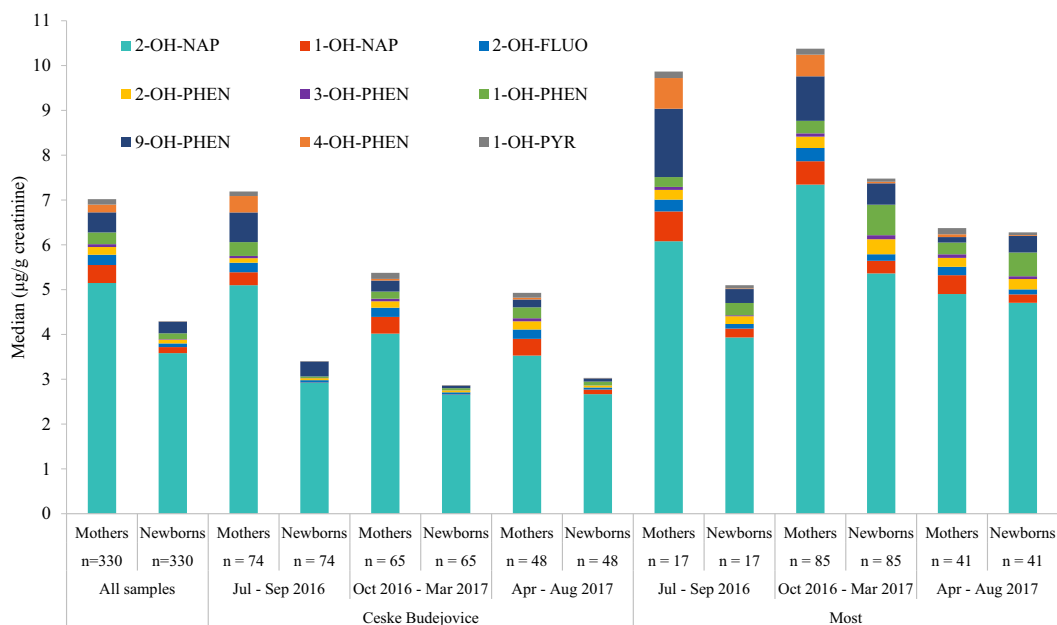**Fig. 1.** Concentrations (μg/g creatinine) of detected OH-PAHs in mothers' and newborns' urine samples.

October 2016 to March 2017 in the same locality (8.88 μg/g creatinine and 6.76 μg/g creatinine), and the median concentration of ΣOH-PAHs in urine samples collected in April–August 2017 (5.82 μg/g creatinine) as shown in Fig. 1. However, as Fig. 2 shows, the obtained concentrations of OH-PAHs in urine do not correlate with PAHs measured in the ambient air. We assume that in this case mothers were exposed to PAHs from other sources, such as diet.

In the case of mothers from the Most region, no statistically significant difference ($\alpha = 0.05$) in the ΣOH-PAHs amount was determined between the first two sampling rounds (July–September 2016 (11.42 μg/g creatinine) and October 2016–March 2017 (12.59 μg/g creatinine)). The median concentration of ΣOH-PAHs collected in the third sampling round (April–August 2017) was approximately 1.5 times lower (8.65 μg/g creatinine) compared to the previous sampling rounds (Fig. 1). This outcome is probably caused again by exposure sources other than the inhalation of PAH-contaminated air (Fig. 2).

For a clear demonstration of the correlation between PAHs present in the ambient air and their monohydroxylated metabolites measured in urine, Fig. 2 only shows the results for phenanthrene (PHEN) (measured in the air) and ΣOH-PHEN (sum of 1-, 2-, 3-, 4- and 9-OH-PHEN) measured in mothers' urine samples. The results for other PAHs are very similar. The detailed results of all PAHs in ambient air are documented in **Table S2** in the Supplementary data. Though Fig. 2 shows that the highest concentration of PHEN in the air was measured in February, it was not the month when the highest ΣOH-PHEN in urine was determined. The same trend was observed for all measured PAHs and their monohydroxylated metabolites in urine. The highest concentrations of PAHs were measured in the air samples collected in both Ceske Budejovice and Most in February 2017 (ΣPAHs 28.2 ng/m³ and 22.0 ng/m³). However, these results do not correspond with the levels of ΣOH-PAHs measured in the urine. According to the highest concentrations of PAHs in the ambient air in Ceske Budejovice in

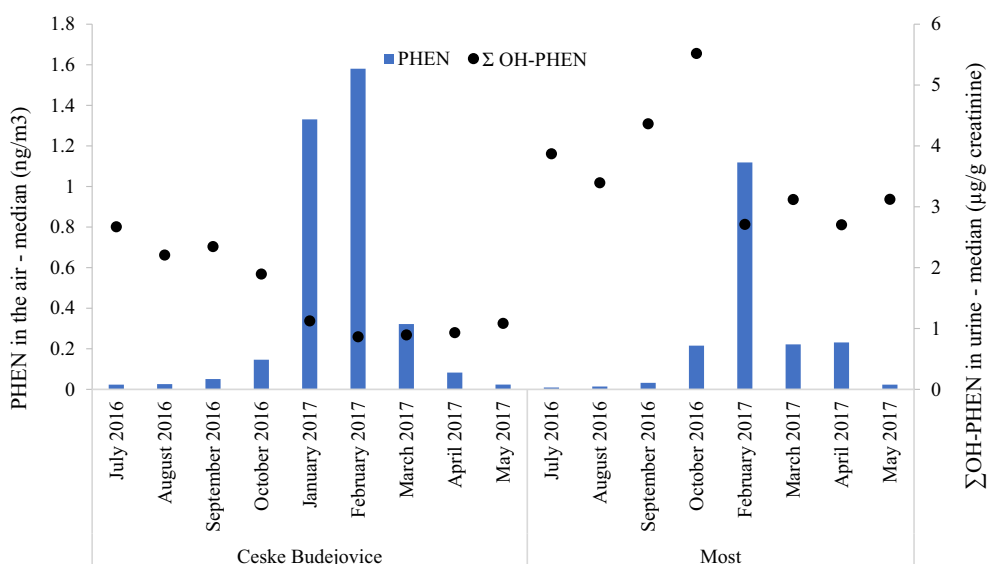**Fig. 2.** Comparison of PHEN concentrations in the air and ΣOH-PHEN measured in urine.

February 2017, the concentrations of OH-PAHs in urine should be the highest as well. However, as shown in Fig. 2, OH-PAH concentrations in urine are the lowest in February 2017. The same trend can be observed in Most, the second sampling locality.

3.2.3. Urine samples collected from newborns born in Ceske Budejovice and Most

In the newborns' urine samples, there were comparable median concentration in samples collected in all three sampling rounds in Ceske Budejovice (July–September 2016 (3.86 μg/g creatinine), October 2016–April 2017 (3.65 μg/g creatinine) and May–August 2017 (3.43 μg/g creatinine) as shown in Fig. 1. The amount of ΣOH-PAHs in newborns' urine samples from Most collected in July–September 2016 (4.96 μg/g creatinine) was statistically significantly lower compared to the second and third sampling rounds (October 2016–April 2017 (8.29 μg/g creatinine), May–August 2017 (8.04 μg/g creatinine) as shown in Fig. 1. Then again, these outcomes do not correlate with the concentration of PAHs measured in the ambient air.

Our results indicate that there is possibly another exposure source that has a stronger influence on the concentrations of OH-PAHs in the urine. It is assumed that diet accounts for almost 80% of the total PAH exposure (Li et al., 2016; Ma and Harrad, 2015). The concentrations of PAHs measured in the whole-day diets from mothers participating in this study will be published in our other paper (Polachova et al., 2020). Unfortunately, breast milk samples were not collected within the Qualitas project.

4. Discussion

Our results were compared with other studies and our previous study, where urine samples collected from mothers and their newborns living in two localities of the Czech Republic (control locality Ceske Budejovice and highly air-polluted Karvina) were measured (Urbancova et al., 2017). As shown in Table 3, the analyte with the highest concentration in all of the studies was 2-OH-NAP, which corresponds with our results from this study and our previous study. The profiles of other target analytes are variable between countries. This result indicates that people in different countries are exposed to different mixtures of PAHs present in the air, diet and other sources.

The concentrations of OH-PAHs in urine samples collected from mothers were comparable with those reported in the US study in 2012 (Fan et al., 2012), the study from India in 2012 (Guo et al., 2013a), where samples of the general population were assessed, and our previously published paper (Urbancova et al., 2017). The concentrations of target compounds in urine samples collected from newborn children were comparable with the samples collected from infants

living in the USA (Dobraca et al., 2018) and the samples collected from newborns which we analysed in our previous study (Urbancova et al., 2017).

5. Conclusions

A total of 660 samples was measured to detect the presence of 11 OH-PAHs in urine samples collected from mothers and their newborns residing in two localities of the Czech Republic, in order to assess exposure to PAHs in this particular part of the Czech population.

The most abundant compound was 2-OH-NAP, which was found in 100% of the samples. It was also the analyte with the highest concentration, which corresponds with the results from other published papers from Poland, USA, Germany, India, and Iran (Ramsauer et al., 2011; Sochacka-Tatara et al., 2018; Dobraca et al., 2018; Kelishadi et al., 2018; Guo et al., 2013a; Fan et al., 2012) and the results from our previous study (Urbancova et al., 2017).

The median concentration of ΣOH-PAHs in all urine samples was approximately 1.7 times higher in urine samples collected from mothers (median concentration 8.96 μg/g creatinine) compared to their newborns (median 5.15 μg/g creatinine). Moreover, the concentration of these compounds in the urine samples collected in Most were almost 2 times higher compared to the samples from Ceske Budejovice (median concentration of ΣOH-PAHs 9.28 μg/g creatinine and 4.92 μg/g creatinine). The most contaminated samples were collected in Most in the period October 2016–March 2017 from both mothers (12.59 μg/g creatinine) and their newborns (8.29 μg/g creatinine).

The results obtained for newborns in Most seem to be similar to our previous results (Urbancova et al., 2017), where urine samples collected in Karvina and Ceske Budejovice were assessed: BaP level in Karvina in the summer period (August–October 2013) corresponded to Ceske Budejovice in the summer and winter periods (January–April 2014), but the median concentration of ΣOH-PAHs in newborn urine samples in Karvina was approximately 2 times higher in summer and 4 times higher in winter. We may assume that the population in the previously highly air-polluted mining districts carries some long-term changes (maybe existing changes in genetic information), which also affect the metabolism of PAHs. It could be related to the long-lasting effect, corresponding to the shortened life expectancy.

Abbreviations

1-OH-NAP
Naphthalene-1-ol
1-OH-PHEN
Phenanthrene-1-ol

Table 3
Comparison of measured concentrations of OH-PAHs in urine in the presented study with other papers.

	USA 2006 Girls[a] Dobraca et al., 2018	Poland 2007 Children[b] Sochacka-Tatara et al., 2018	Germany 2010 Smokers[c] Ramsauer et al., 2011	USA 2012 General population[a] Fan et al., 2012	India 2012 General population[a] Guo et al., 2013a	Iran 2016 Children[a] Kelishadi et al., 2018	Czech Republic 2013–2014 Women[d] Urbancova et al., 2017	Czech Republic 2013–2014 Newborns[d] Urbancova et al., 2017	Czech Republic 2016–2017 Women[d] [Presented study]	Czech Republic 2016–2017 Newborns[d] [Presented study]
Samples	n = 431	n = 218	n = 100	n = 34	n = 38	n = 150	n = 265	n = 266	n = 330	n = 330
1-OH-NAP	1.19	3.30	4.26	1.59	1.10	0.36	0.73 (0.54)	0.23 (0.45)	0.36 (0.41)	0.12 (0.20)
2-OH-NAP	2.16	8.20	8.47	7.65	3.80	0.42	5.98 (5.36)	2.01 (3.10)	4.66 (5.16)	2.28 (3.60)
2-OH-FLUO	0.20	0.91	1.56	0.87	0.35	n.a.	0.41 (0.37)	0.12 (0.18)	0.23 (0.23)	0.07 (0.11)
Σ OH-PHEN	0.26	1.28	0.56	1.76	0.73	0.11	1.19 (0.99)	0.60 (0.88)	1.15 (1.24)	0.41 (0.76)
1-OH-PYR	0.10	0.36	0.16	0.99	0.42	0.10	0.21 (0.21)	0.06 (0.10)	0.12 (0.13)	0.05 (0.10)
6-OH-CHRY	n.a.	n.a.	n.a.	n.a.	< 0.01	n.a.	< 0.01	< 0.01	< 0.01	< 0.01
3-OH-BaP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	< 0.90	< 0.90	< 0.90	< 0.90
Σ OH-PAH	3.92	15.96	15.00	12.86	6.40	0.99	9.55 (8.14)	3.19 (4.99)	7.50 (8.66)	2.97 (4.83)

a – Geometric mean (ng/mL urine).
b – Median (μg/g creatinine).
c – Median (ng/mL urine).
d – Median (ng/mL urine; μg/g creatinine).

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
ADHD   Attention deficit hyperactivity disorder
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
ESI-   Electrospray ionization (negative mode)
HiVol samplers   High-Volume air samplers
IARC   International Agency for Research on Cancer
IPCS   International Programme on Chemical Safety
JECFA   Joint FAO/WHO Expert Committee on Food Additives
LLE   Liquid-liquid extraction
LOQ   Limit of quantification
NIST   National Institute of Standards and Technology
OH-PAHs   Monohydroxylated polycyclic aromatic hydrocarbons
PAHs   Polycyclic aromatic hydrocarbons
PHEN   Phenanthrene
PFP   Pentafluorophenyl
SCF   Scientific Committee on Food
SRM   Standard Reference Material
UHPLC–MS/MS
Ultra-high performance liquid chromatography coupled with tandem mass spectrometry

Consent for publication

Author and all co-authors agree with the publication of this article.

Ethical approval and consent to participate

The study was approved by the Ethics Committee of Ceske Budejovice Hospital, Department of Obstetrics and Department of Neonatology, the Most Hospital, Department of Obstetrics and Department of Neonatology and the Institute of Experimental Medicine of the CAS in Prague. Each mother signed a written consent to participate in the study.

Availability of supporting data

Supporting data are available for download as Supplementary information or available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Katerina Urbancova: Investigation, Validation, Writing - original draft.**Darina Dvorakova:**Investigation, Validation.**Tomas Gramblicka:** Investigation, Validation.**Radim J. Sram:**Conceptualization.**Jana Hajslova:**Conceptualization.**Jana Pulkrabova:**Conceptualization, Writing - original draft.

Declaration of competing interest

All authors declare that they have no competing interests.

Appendix A. Supplementary data

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