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Possible relationship between respiratory diseases and urinary concentrations of polycyclic aromatic hydrocarbon metabolites – a pilot study

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Abstract
This study investigates the potential relationship between exposure to polycyclic aromatic hydrocarbons (PAHs), specifically monohydroxylated metabolites (OH-PAHs), in urine, and the prevalence of respiratory diseases in 2-year-old children residing in two locations within the Czech Republic – České Budějovice (control location) and the historically contaminated mining district of Most. Despite current air quality and lifestyle similarities between the two cities, our research aims to uncover potential long-term health effects, building upon previous data indicating distinctive patterns in the Most population.

A total of 248 urine samples were analysed for the presence of 11 OH-PAHs. Employing liquid-liquid extraction with ethyl acetate and clean-up through dispersive solid-phase extraction, instrumental analysis was conducted using ultra-high performance liquid chromatography coupled with tandem mass spectrometry. The incidence of respiratory diseases was assessed through questionnaires administered by paediatricians.

The concentrations of OH-PAHs were elevated in urine samples from 2-year-olds in Most compared to those from České Budějovice. The incidence of respiratory diseases showed statistically significant higher levels of OH-PAHs in children from Most, together with a higher incidence of influenza. This association underlines the impact of environmental PAH exposure on children's respiratory health. It suggests that elevated urinary OH-PAH levels indicate an increased risk of developing respiratory diseases in the affected population. Further studies are needed to clarify the possible long-term health effects and to contribute to sound public health strategies.

Keywords: Influenza; Monohydroxylated PAH metabolites; Polycyclic aromatic hydrocarbons; Respiratory diseases; Urine; 2-year-old toddlers

Highlights:
- The concentration of OH-PAH metabolites in urine was found in 100% of samples.
- Higher OH-PAH levels in Most vs. České Budějovice.
- Increased respiratory diseases in Most, with influenza showing location-based differences.
- External pollution is linked to elevated OH-PAH levels; in utero PAH exposure affects lung development.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are non-polar environmental contaminants formed during incomplete combustion or pyrolysis of organic matter (Guo et al., 2012; Jain, 2020). Due to their ubiquitous presence in the environment, people can be easily exposed to these compounds, mainly through the digestion of contaminated foods or inhalation of polluted air or cigarette smoke (Li et al., 2016). Occupationally exposed individuals (firefighters, asphalt workers, and coke-oven workers) can also be significantly exposed to PAHs by transfer through the skin (Oliveira et al., 2017; Onyemauwa et al., 2009).

PAHs are generally not considered to be bioaccumulative substances due to their relatively rapid metabolism (excretion

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half-life of 2–6 hours) in the human body. However, their risk to human health increases significantly after undergoing metabolic transformation. PAH metabolites, particularly reactive epoxides, have the potential to form adducts with DNA, leading to carcinogenesis (Taioli et al., 2007). Another critical consequence of PAH exposure is the generation of reactive oxygen species, which induce oxidative stress in cells and contribute to DNA damage, including the production of 8-hydroxy-2´-deoxyguanosine (8-OHdG) (Peng et al., 2020; Stading et al., 2021).

Prenatal exposure to PAHs has been associated with a number of adverse health outcomes, including low birth weight, developmental delay, cognitive impairment and an increased risk of asthma in childhood. Exposure during pregnancy can have a direct effect on the developing foetus because PAHs can cross the placental barrier. Studies have shown that PAHs can disrupt normal endocrine function, affect foetal development, and possibly cause genetic mutations (Drwal et al., 2019; Jedrychowski et al., 2014; Perera et al., 2005).

The presence of PAH metabolites in the uterus has been associated with the dysregulation of lung development, resulting in early respiratory symptoms after birth (Jedrychowski et al., 2015; Karimi et al., 2015; Lag et al., 2020). Jedrychowski et al. (2005) observed an increased risk of barking cough, wheezing, sore throat, and ear infection following prenatal exposure. Immunotoxic PAHs during pregnancy have been linked to impaired immune function, potentially contributing to the elevated susceptibility of newborns and young infants to respiratory infections.

The impact of PAH exposure on respiratory diseases is elucidated by three primary physiological mechanisms: systemic oxidative stress, epithelial dysfunction, and immune disturbance (Lag et al., 2020). Understanding these complex pathways is crucial for comprehending the intricate relationship between PAH exposure and the development of respiratory disease.

However, not all of the PAH metabolites result in producing DNA adducts. The reactive epoxides can be transformed into hydroxylated metabolites (OH-PAHs) that are in the second phase of the metabolism conjugated with glucuronic acid, sulphates, or glutathione to produce more water-soluble compounds that can be excreted from the body via urine (Abdel-Shafy and Mansour 2016; Oliveira et al., 2017). In such biological material, these metabolites can then be reliably measured.

Given the potential health risks of exposure to PAHs, it is essential to minimise exposure, especially in pregnant women and children, to protect their health and development. This includes implementing measures to reduce emissions of PAHs to the environment and minimising personal exposure to sources of PAHs.

In our previous study (Urbancova et al., 2020), we observed higher concentrations of urinary OH-PAHs of newborns from Most compared to the second sampling location České Budějovice. Still, the concentrations of benzo[a]pyrene (BaP) in ambient air in both locations were similar. Therefore, we hypothesised that the population of highly polluted mining districts 40–50 years ago can carry some long-term changes (maybe changes in the genetic information), which also affect the metabolism of PAHs (Sram, 2020).

This study aimed to evaluate if there is any relationship between the levels of OH-PAHs in urine and respiratory diseases in two cities of the Czech Republic – the control location České Budějovice and the previously highly polluted mining district Most. Furthermore, we tried to find the cause of the differences in PAH exposure by investigating the information provided in personal questionnaires. The district of České Budějovice city in Southern Bohemia was selected as a control locality, according to our previous studies (Sram et al., 2016; Veleminsky et al., 2016). In the 1970s and 1980s, the district of Most city in Northern Bohemia was characterised by significant air pollution due to power plants and local heating emissions, which used brown coal with a high content of sulphur.

Materials and methods

Certified standards and materials

Certified standards of naphthalene-1-ol (1-OH-NAP) and naphthalene-2-ol (2-OH-NAP) were bought from Absolute Standards, Inc. (USA); phenanthrene-3-ol (3-OH-PHEN), phenanthrene-9-ol (9-OH-PHEN), pyrene-1-ol (1-OH-PYR), and benzo[α]pyrene-3-ol (3-OH-BaP) were supplied by Neochema (Germany), and chrysene-6-ol 6-OH-CHRY was purchased from AccuStandard® (USA). Analytes fluorene-2-ol (2-OH-FLUO), phenanthrene-1-ol (1-OH-PHEN), phenanthrene-2-ol (2-OH-PHEN), phenanthrene-4-ol (4-OH-PHEN), and isotopically labelled analogues of target compounds, specifically d_7-1-OH-NAP, d_7-2-OH-NAP, d_9-2-OH-FLUO, d_9-1-OH-PHEN, d_9-2-OH-PHEN, d_9-3-OH-PHEN, d_8-9-OH-PHEN, d_9-1-OH-PYR, and d_{11}-3-OH-BaP were obtained from Toronto Research Chemicals Inc. (Canada). Creatinine was bought from Sigma-Aldrich (USA). All of the supplied standards and their isotopically labelled analogues were of at least 98% purity. All mixtures were prepared in methanol and stored in the freezer (–20 °C). The Standard Reference Material® 3673 (Organic Contaminants in Non-Smokers' Urine) (SRM 3673) was delivered by the US National Institute of Standards and Technology (NIST, USA).

Chemicals, reagents, and other materials

Sorbent SupelTM QuE Z-Sep, enzyme β-glucuronidase (type HP-2, glucuronidase activity ≥100 000 units/ml, sulfatase activity ≤7 500 units/ml), ethyl acetate, picric acid, and polypropylene centrifuge tube filters (nylon, pore size 0.22 μm) were purchased from Sigma-Aldrich (USA). Methanol (HPLC gradient) was obtained from Merck (Germany). Unsterile polyvinylidene fluoride (PVDF) filters (0.22 μm, Ø 33 mm) were bought from Rotilabo® (Germany), and 96-well microplates were supplied by the Gama Group (Czech Republic).

Sample collection

The urine samples were collected within the grant of the Czech Health Agency. The urine was sampled in two locations in the Czech Republic, České Budějovice and Most, from 2-year-old children. All urine samples were collected as spot samples. The study was approved by the Ethics Committee of both hospitals (reference number 5/16) and the Institute of Experimental Medicine of the CAS in Prague. Each mother signed the approval to include their child in the study. A total of 248 samples (130 samples from children from České Budějovice and 118 samples from children living in Most) were collected. The urine collection was carried out from February 2019 to December 2020 together with a questionnaire provided by the paediatricians, which included information such as disease incidence, length of breastfeeding, child's development (height, weight, head circumference), smoking habits in the family, and a type of household heating (Table 1).

Table 1. Study demographic overview

Parameter			All (n = 248)	České Budejovice (n = 130)	Most (n = 118)
			Mean ± SD	Mean ± SD	Mean ± SD
Age	Mother	years	33.6 ± 5.1	34.2 ± 4.7	33.1 ± 5.4
	Child	years	2.7 ± 0.5	2.6 ± 0.6 *	2.8 ± 0.4 *
Breast feeding	Full	months	6.7 ± 5.2	6.2 ± 4.2	7.3 ± 6.1
	Partial	months	12.7 ± 8.0	12.2 ± 7.1	13.2 ± 8.9
Maternal smoking	Smoking	%	10	6 +	14 +
	Before pregnancy	%	24	16 ++	32 ++
	ATS – pregnancy	cig/day	0.3 ± 1.1	0.2 ± 1.0	0.4 ± 1.2
	ATS – 1st year	cig/day	0.6 ± 1.8	0.4 ± 1.5	0.7 ± 2.0
	ATS – 2nd year	cig/day	1.6 ± 3.6	1.6 ± 3.8	1.7 ± 3.4
	ETS – pregnancy	cig/day	2.9 ± 5.8	1.6 ± 4.8 *	4.3 ± 6.6 *
	ETS – 1st year	cig/day	3.0 ± 5.9	1.7 ± 4.8 *	4.4 ± 6.7 *
	ETS – 2nd year	cig/day	3.0 ± 5.9	1.8 ± 4.8	4.4 ± 6.8
Heating type	Remote heating	%	34	24 +++	46 +++
	Gas heating out of flat	%	9	10	8
	Gas heating in flat	%	12	14	10
	Coal heating out of flat	%	7	11 +	3 +
	Coal heating in flat	%	0	1	0
	Electric heating	%	14	20 ++	7 ++
	Fireplace in flat	%	10	12	8
Parent's education (years) mother / father	<9	%	1 / 0	0 / 0	1 / 0
	9	%	7 / 6	2 / 2	14 / 10
	12	%	15 / 30	14 / 24	16 / 37
	13	%	32 / 35	27 / 39	38 / 29
	Still student	%	0 / 0	0 / 1	1 / 0
	≥16	%	45 / 30	56 / 34	30 / 23
Length	At birth	cm	50.0 ± 1.8	49.6 ± 1.8 ***	50.4 ± 1.8 ***
	18 months	cm	82.8 ± 3.4	83.6 ± 3.6 ***	81.9 ± 2.9 ***
Weight	At birth	kg	3.4 ± 0.5	3.4 ± 0.5	3.4 ± 0.5
	18 months	kg	11.2 ± 1.4	11.4 ± 1.4	11.0 ± 1.3
Head circumference	At birth	cm	34.5 ± 1.4	34.6 ± 1.3	34.4 ± 1.5
	18 months	cm	47.8 ± 1.5	47.9 ± 1.5 *	47.6 ± 1.6 *

Note: Results of Mann–Whitney U test compared by region * $p < 0.05$; ** $p < 0.01$; ***; $p < 0.001$; logistic regression by region + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$; SD – standard deviation; ATS – Active Tobacco Smoking; ETS – Environmental Tobacco Smoke.

The study enrolled children who had been investigated during delivery (Urbancova et al., 2020). Their gestational age was 35–42 weeks, with birth weights over 3 kg and no gestational defects (Urbancova et al., 2020).

Measurement of urinary creatinine

Concentrations of creatinine were used to correct the different dilutions of each spot of urine sample, and it was measured using a Jaffe's spectrophotometric method, previously described in more detail in a study by Lankova et al. (2016).

Analytical method for the determination of urinary OH-PAHs

Extraction

The samples were extracted using LLE (extraction solvent ethyl acetate) and a clean-up step (Z-Sep sorbent), as described in more detail in our previous paper (Lankova et al., 2016).

Analytical instrumentation

The analysis of 11 OH-PAHs was performed using a UHPLC-MS/MS system 1290 Infinity II (Agilent) liquid chro-

matograph coupled with a QTRAP® 6500+ (SCIEX) mass spectrometer with electrospray ionisation in a negative ion mode (ESI-) with a capillary voltage –4,5 kV and desolvation temperature 500 °C (MRM transitions with corresponding MS parameters are listed in Table S1 in Supplementary data). Analytes were separated on a PFP (pentafluorophenyl) Kinetex column (Phenomenex) with the dimensions 100 mm × 2.1 mm × 1.7 µm. More details about the LC conditions can be found in the paper by Lankova et al. (2016).

Quality assurance/quality control and validation

The validation of the method for creatinine determination is described in detail in our previous study (Lankova et al., 2016). The validation of 11 OH-PAHs in urine was performed using SRM 3673 in six parallels. Solvent calibration was used for the quantification of target analytes. Limits of quantification (LOQs), recovery and repeatability (RSD – relative standard deviation) are summarised in Table 2. Monohydroxylated metabolites of chrysene (CHRY) 6-OH-CHRY and BaP (3-OH-BaP) that were not certified in this material were validated by artificial contamination of a blank urine sample that had been previously tested based on the presence of the target analytes. The used concentration levels were 0.1 ng/ml urine for 6-OH-CHRY and 1 ng/ml for 3-OH-BaP. Recovery for 6-OH-CHRY was 95% with RSD 13% and LOQ 0.01 ng/ml urine, and for 3-OH-BaP 97% with RSD 16% and LOQ 0.90 ng/ml urine. Each day, approximately 20 urine samples were analysed, and with each batch of samples, one duplicate sample was prepared.

Table 2. LOQs, recovery and RSD for the target analytes (n = 6)

Analyte	LOQ (ng/ml urine)	Certified value (ng/ml urine)		Measured value (ng/ml urine)		Recovery (%)	RSD (%)
		Mean	Uncertainty	Mean	Uncertainty		
2-OH-NAP	0.025	1.35	0.03	1.43	0.09	106	6
1-OH-NAP	0.025	211	34	146	12	70	8
2-OH-FLUO	0.025	0.107	0.007	0.106	0.016	99	15
2-OH-PHEN	0.010	0.0247	0.0043	0.0267	0.0041	108	15
3-OH-PHEN	0.010	0.0276	0.0014	0.0278	0.0030	101	11
1-OH-PHEN	0.010	0.0488	0.0075	0.0514	0.0063	105	12
9-OH-PHEN	0.010	0.0116	0.0009	0.0134	0.0015	115	11
4-OH-PHEN	0.010	0.0104	0.0010	0.0088	0.0005	84	5
1-OH-PYR	0.025	0.0305	0.0018	0.0307	0.0023	101	8

Respiratory disease diagnosis

Respiratory diseases were diagnosed during multiple clinic visits, based on the child's clinical symptoms and International Statistical Classification of Diseases 10th Edition (ICD-10): acute nasopharyngitis (J00), acute sinusitis (J01), acute pharyngitis (J02), acute tonsillitis (J03), acute laryngitis and tracheitis (J04), acute upper respiratory infections of multiple and unspecified sites (J06), influenza due to unidentified influenza virus (J11), pneumonia, unspecified organism (J18), acute bronchitis (J20), other chronic obstructive pulmonary disease (J44), asthma bronchiale (J45).

Statistical evaluation

To compare the differences between the concentrations of OH-PAHs in urine, statistical t-test was performed. ICD codes of diagnoses have been collected from paediatric documentation filtered for apparent duplicities, normalised to shortened 2-digit code form for better granularity of the tested data, and then counted per individual child. Mann–Whitney U test (Mann and Whitney, 1947) was used to test the difference between both regions for individual children per individual diagnoses and for the total sum of respiratory diseases, the same as for demographical population parameters. Logistical regression was used to estimate the differences between categorical population parameters such as education. For the estimation of the relation between OH-PAHs and the count of diagnoses, linear regression was calculated on log-transformed values.

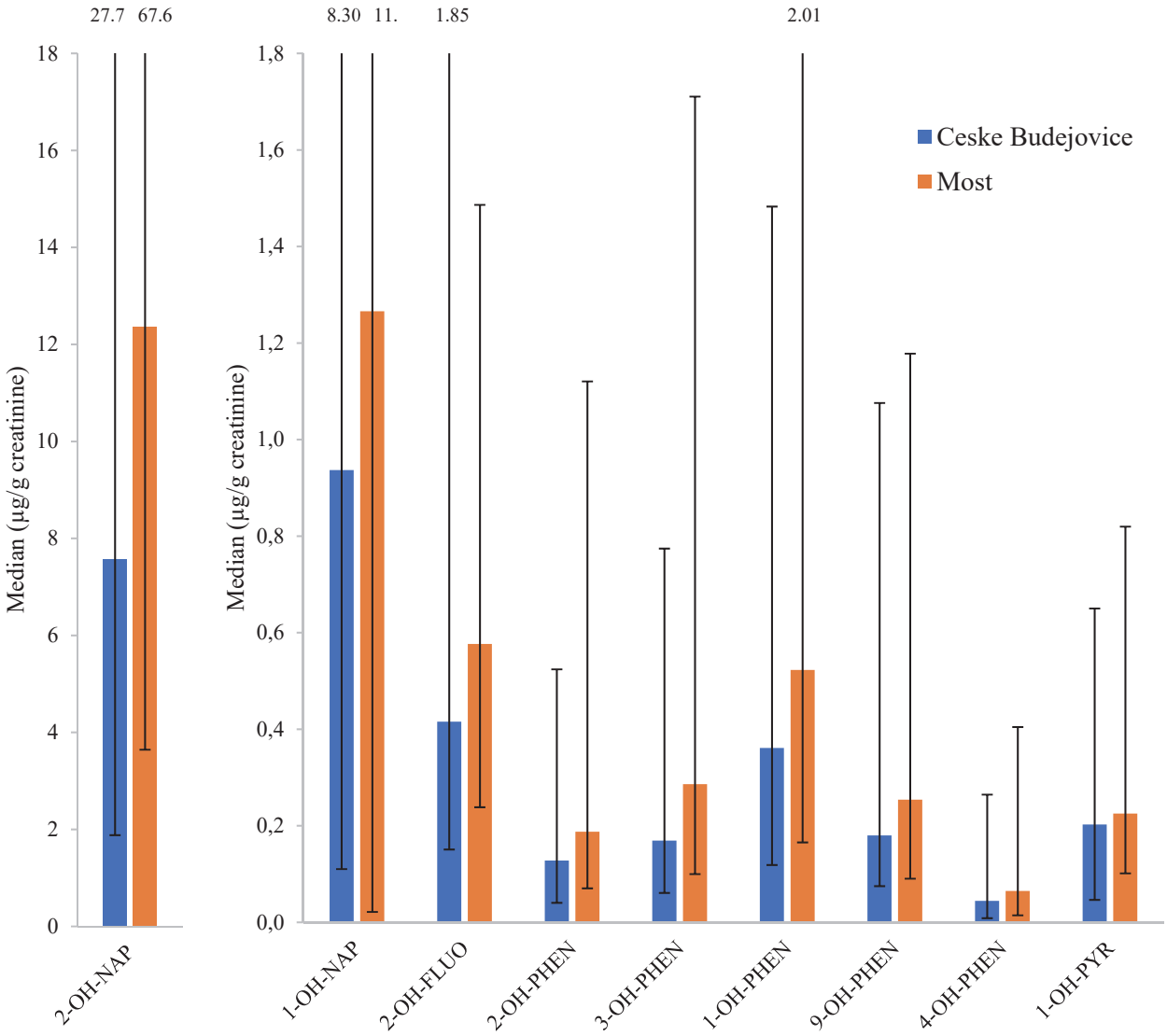
Results

Table 1 summarises information about mothers and children, including information about potential prenatal exposure via the mother's smoking (postnatal information is also available) and home heating type, as well as toddlers' growth up to 18 months.

Analysis of OH-PAHs in urine

The analytes found in all of the measured samples (100%) were 2-OH-NAP, 2-OH-FLUO, 1-OH-PHEN, 3-OH-PHEN, and 9-OH-PHEN, followed by 2-OH-PHEN (99%), 1-OH-NAP (98%), 1-OH-PYR (96%), and 4-OH-PHEN (88%) in urine samples obtained from children from České Budějovice. As far as urine samples collected in Most are concerned, the analytes measured in all of the urine samples (100%) were 2-OH-NAP, 2-OH-FLUO, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 9-OH-PHEN, and 1-OH-PYR, followed by 4-OH-PHEN (96 %) and 1-OH-NAP (93%). In terms of concentration, the analyte with the highest concentration in urine samples collected both in České Budějovice and Most was 2-OH-NAP, with a median concentration 7.6 µg/g creatinine in the urine samples collected in České Budějovice and 12.4 µg/g creatinine in the urine samples obtained in Most.

When comparing the concentrations of target analytes in urine between the locations, it can be observed that median



µg/g creatinine		2-OH-NAP	1-OH-NAP	2-OH-FLUO	2-OH-PHEN	3-OH-PHEN	1-OH-PHEN	9-OH-PHEN	4-OH-PHEN	1-OH-PYR
CB n=130	**Median**	**7.56**	**0.937**	**0.416**	**0.128**	**0.169**	**0.361**	**0.180**	**0.044**	**0.203**
	5th Percentile	1.88	0.110	0.151	0.040	0.061	0.119	0.075	0.009	0.046
	95th Percentile	27.7	8.30	1.85	0.524	0.774	1.48	1.08	0.265	0.650
MOST n=118	**Median**	**12.4**	**1.27**	**0.577**	**0.188**	**0.286**	**0.523**	**0.254**	**0.065**	**0.225**
	5th Percentile	3.64	0.022	0.238	0.070	0.100	0.166	0.090	0.015	0.101
	95th Percentile	67.6	11.1	1.49	1.12	1.71	2.01	1.18	0.405	0.820

Fig. 1. Median concentrations of target OH-PAHs in urine of a 2-year-old. The error bars present 5th and 95th percentiles.

concentrations for the OH-PAHs measured in urine are statistically significantly ($p < 0.05$) higher in samples collected in Most. The median concentration of the sum of all measured OH-PAHs in urine (ΣOH-PAHs) was 20.2 µg/g creatinine in samples collected in Most and 11.4 µg/g creatinine in urine samples collected in České Budějovice. The trend of higher median concentration can be seen in all target analytes (Fig. 1).

Comparison of urinary OH-PAHs of newborns and 2-year-old children

The 2-year-old children from whom the urine samples in this study were collected were also sampled as newborns in our previous study (Urbancova et al., 2020). Fig. 2 compares the median concentrations of OH-PAHs in the urine of newborns at the age of two years. The same trend of higher levels of OH-PAHs in samples from Most can be observed for both newborns and 2-year-old children. The median concentration of ΣOH-PAHs in urine samples of newborns from Most was 8.1 µg/g creatinine, whereas from České Budějovice it was 3.8 µg/g creatinine.

Respiratory diseases

When we analysed the total incidence of respiratory diseases (J00–J99) in our cohorts of 2-year-old children from Most and

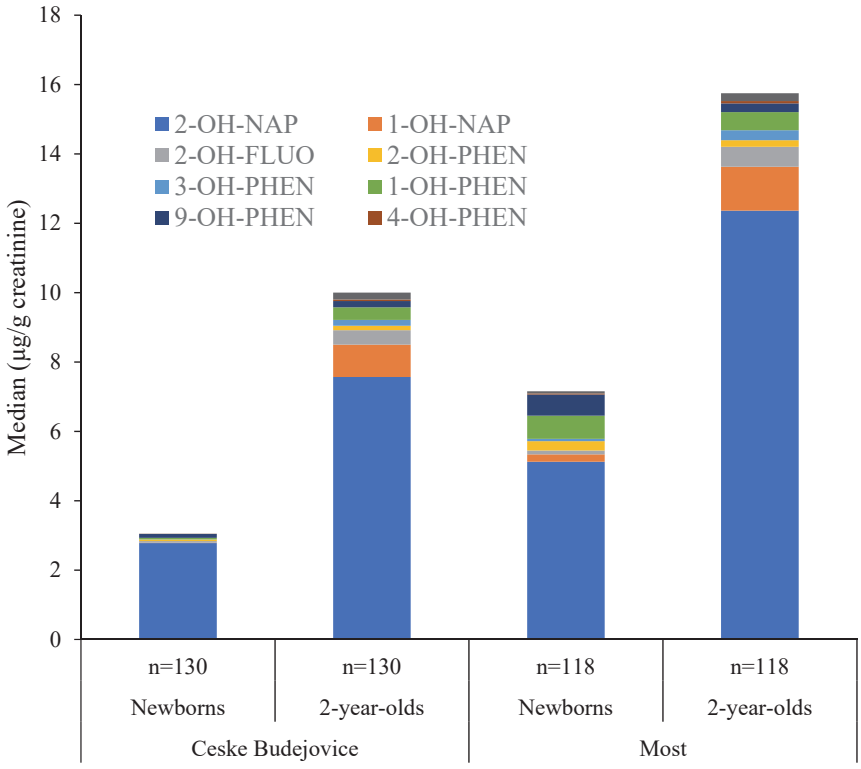


Fig. 2. Comparison of the median concentrations of target OH-PAHs in the urine of newborns and 2-year-old children

České Budějovice, they were significantly higher in Most ($p < 0.05$); specifically in the diagnosed influenza due to unidentified influenza virus (J11; $p < 0.001$) (Table 3). No differences between localities for other respiratory diseases, especially bacterial diseases, have been demonstrated.

The association between morbidity of respiratory diseases and OH-PAH

The relation of OH-PAH in urine and the incidence of respiratory diseases was estimated after logarithmic normalisation. We found that Respiratory diseases (J00–J99) and Acute pharyngitis (J02) are significantly associated with OH-PAHs levels in urine (Table 4). We also tested multivariate models adjusted to locality, family smoking history, and education (Table 5). A statistically significant relation between adjusted respiratory diseases and OH-PAH was only observed for Acute pharyngitis (J02).

Table 3. Frequency of illnesses

ICD-10	Disease	All (n = 248)	České Budějovice (n = 130)	Most (n = 118)
		Mean ± SD	Mean ± SD	Mean ± SD
J00	Acute nasopharyngitis (common cold)	1.08 ± 1.43	0.96 ± 1.39	1.22 ± 1.46
J01	Acute sinusitis	0.02 ± 0.17	0.02 ± 0.12	0.03 ± 0.21
J02	Acute pharyngitis	0.34 ± 0.89	0.32 ± 0.85	0.36 ± 0.93
J03	Acute tonsillitis	0.20 ± 0.50	0.18 ± 0.48	0.21 ± 0.52
J04	Acute laryngitis and tracheitis	0.31 ± 0.69	0.27 ± 0.67	0.35 ± 0.71
J06	Acute upper respiratory infections of multiple and unspecified sites	0.71 ± 1.19	0.71 ± 1.05	0.70 ± 1.34
J11	Influenza due to unidentified influenza virus	0.25 ± 0.75	0.05 ± 0.29	0.47 ± 1.00 ***
J18	Pneumonia, unspecified organism	0.01 ± 0.11	0.01 ± 0.09	0.02 ± 0.13
J20	Acute bronchitis	0.50 ± 1.02	0.50 ± 1.09	0.50 ± 0.94
J44	Other chronic obstructive pulmonary disease	0.02 ± 0.13	0.03 ± 0.17	0.00 ± 0.00
J45	Asthma	0.02 ± 0.21	0.02 ± 0.12	0.03 ± 0.28
J00–J99	Respiratory diseases	3.21 ± 2.60	2.92 ± 2.50	3.53 ± 2.68 *

Note: Results of Mann–Whitney U test compared by region * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 4. Relationship between OH-PAH and respiratory diseases

		2-OH-NAP	1-OH-NAP	2-OH-FLUO	2-OH-PHEN	3-OH-PHEN	1-OH-PHEN	9-OH-PHEN	4-OH-PHEN	1-OH-PYR
J00	Acute nasopharyngitis (common cold)	–0.01	0.01	0.00	0.02	–0.02	0.01	0.03	0.05	0.02
J01	Acute sinusitis	0.10	–0.02	–0.10	–0.15	–0.06	–0.08	–0.06	–0.13	–0.18 *
J02	Acute pharyngitis	–0.03	0.03	0.16 *	0.23 **	0.22 **	0.26 ***	0.17 *	0.15	0.21 **
J03	Acute tonsillitis	0.04	0.05	0.00	–0.03	–0.02	–0.07	–0.04	–0.03	0.02
J04	Acute laryngitis and tracheitis	0.08	–0.19 *	0.05	0.00	0.08	0.02	0.02	–0.07	–0.04
J06	Acute upper respiratory infections of multiple and unspecified sites	0.15 *	0.09	0.19 *	0.10	0.12	0.11	0.11	0.10	0.11
J11	Influenza due to unidentified influenza virus	0.18 *	–0.01	–0.01	0.04	0.05	0.02	0.11	0.05	0.06
J18	Pneumonia, unspecified organism	–0.01	0.03	–0.04	0.07	0.00	0.03	0.03	0.04	0.05
J20	Acute bronchitis	–0.01	–0.05	0.02	0.10	0.18 *	0.15 *	0.12	0.17 *	0.03
J44	Other chronic obstructive pulmonary disease	–0.08	–0.04	–0.14	–0.18 *	–0.12	–0.17 *	–0.08	–0.03	–0.13
J45	Asthma	–0.03	–0.05	–0.06	–0.13	–0.06	–0.09	–0.05	–0.02	–0.09
J00–J99	Respiratory diseases	0.13	0.00	0.17 *	0.16 *	0.21 **	0.17 *	0.19 *	0.17 *	0.15

Note: Beta coeficient results of Regression * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 5. Relationship between OH-PAH and respiratory diseases – adjusted

		2-OH-NAP	1-OH-NAP	2-OH-FLUO	2-OH-PHEN	3-OH-PHEN	1-OH-PHEN	9-OH-PHEN	4-OH-PHEN	1-OH-PYR
J02	Acute pharyngitis	0.83	0.58	1.02	1.77	2.11 *	2.19 *	1.07	1.14	1.34
J00–J99	Respiratory diseases	1.23	0.58	1.77	1.59	1.81	1.22	1.60	1.42	1.21

Note: Logistic regression OddR * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

Our objective was to assess the potential relationship between urinary levels of OH-PAHs and the incidence of respiratory diseases in 2-year-old children in two distinct Czech cities which have varying degrees of air pollution – namely, the control location České Budějovice and the formerly heavily polluted mining district of Most. Additionally, we tried to investigate whether differences in PAH exposure were associated with information obtained from personal questionnaires.

The idea of investigating the association is based on epidemiological and laboratory studies reporting that PAHs cause respiratory distress (Gerde et al., 1993). The risk of death from respiratory disease, including obstructive lung disease or asthma, is increased in children exposed to PAHs (Barraza-Villarreal et al., 2014). The contribution to the effect of PAH exposure on infectious respiratory diseases remains unclear.

The district of Most is an often studied area where intensive mining and combustion of brown coal in the Northern Bohemian basin has resulted in high concentrations of air pollutants compared to the controlled locality of CB. Most has a broad background in human biomonitoring aimed at assessing the impact of air on human health (Sram et al., 1996).

Our study involved a comparison of children born and residing in two geographically and industrially/agriculturally diverse localities within the Czech Republic. The selected locations (České Budějovice and Most) differed not only in their geographical characteristics but also in the prevailing industrial and agricultural activities, providing a comprehensive examination of the potential impact of these factors on OH-PAH levels and respiratory health outcomes. The levels of environmental pollutants in 2019 and 2020 (i.e., the time of urine collection) obtained from the Czech Hydrometeorological Institute are presented. The level of PM 2.5 followed a similar pattern in both localities over the years (Fig. 3). However, Most presented a higher level of PM 2.5 (CHMI, © 2022a). Data on the concentration of BaP is not available for Most; therefore, the interpolated yearly average level measured in surrounding CHMI stationary stations is presented. The average annual level of BaP in Most corresponds to 1 ng/m^3, while it reaches the level of 0.4 ng/m^3 in České Budějovice (CHMI, © 2022b). We assumed that, due to exposure, respiratory disease may have developed in the subjects residing in Most. Furthermore, in agreement with previously published research (Chen et al., 2023), the significantly higher incidence of diagnosed influenza in Most can be linked to the occurrence of other air pollutants such as NO_2, see Fig. S2.

Another important source of personal exposure is indoor air-pollution in a household. Household indoor air-pollution is, among other things, strongly affected by the heating system, especially during the winter season. There are several types of heating in Czech households. Table 1 presents a scattering of heating methods among the households of tested children. The main differences between the two types are the following: District heating delivers heat through insulated pipes, minimising

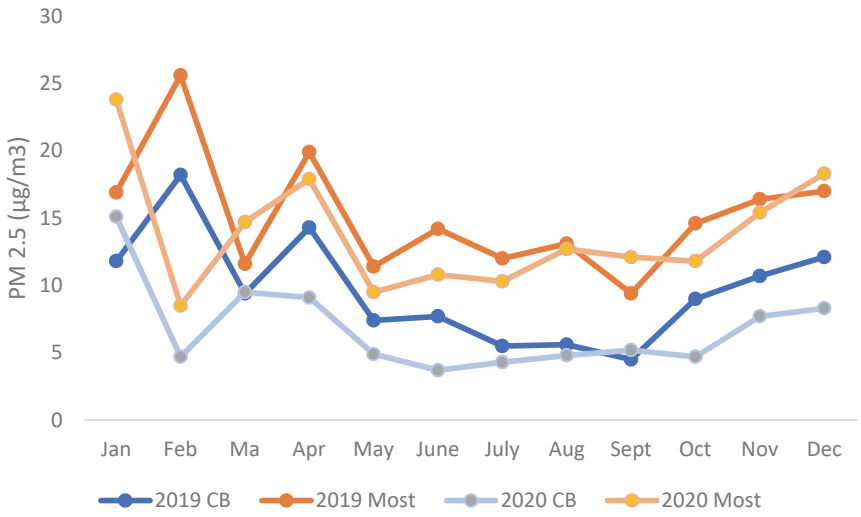


Fig. 3. Levels of PM 2.5 in the studied localities of Most and České Budějovice (CB) in 2019 and 2020 (CHMI, © 2022a, b)

indoor pollution. Gas heating, which is cleaner than coal, still emits pollutants when burned outside the home. Coal heating releases high levels of particulate matter and PAHs, which significantly impact air quality and health, increasing the risk of respiratory problems.

OH-PAH in urine measured in newborns with expected prenatal exposure to air pollution from similar locations in the Czech Republic has recently been associated with neonatal growth parameters (Sram et al., 2024). Fig. 2 shows the comparison between the median concentrations of OH-PAHs in the urine of newborns (Urbancova et al., 2020) and at the age of 2 years. The same trend of higher levels of OH-PAHs in samples from Most was observed at both time-points.

Data provided by questionnaires was carefully studied to explain this outcome. Specifically, the impact of breastfeeding, type of household heating, and smoking habits in the family were investigated. However, it was concluded that breastfeeding did not significantly impact the levels of OH-PAHs in urine because children from both České Budějovice and Most were breastfed for a similar duration. It is well-known that predominant breastfeeding for at least six months and partial breastfeeding for up to one year may reduce the prevalence and subsequent morbidity of respiratory illness and infection in infancy (Oddy et al., 2003). Our finding of very slight differences in the morbidity of respiratory diseases (except for influenza) may also suggest that there was no significant difference in the duration of breastfeeding divided into several time categories (data not shown).

We saw the same outcome when evaluating the type of household heating; both of these parameters were very similar in České Budějovice and Most. The only slight difference was observed in smoking habits in the family. When investigating the smoking habits of the mothers, we concluded that in Most, more mothers reported smoking before the pregnancy (32%) compared to the mothers from České Budějovice (16%). However, after the pregnancy, the number of smoking mothers was lower both in Most (14%) and České Budějovice (6%) (Table 1). The mean number of cigarettes smoked during the day by the mothers (ATS Table 1) was comparable between the locations, as well as the number of fathers who smoked before and after the pregnancy of their partner.

Given the elevated concentration of OH-PAHs in the urine of newborns from Most at the time of delivery, our findings lend support to the hypothesis proposed by Jedrychowski et al. (2015) that in utero exposure to PAH metabolites may lead to the dysregulation of lung development, resulting in early respiratory symptoms after birth. Furthermore, in line with another hypothesis by Jedrychowski et al. (2005), prenatal exposure to immunotoxic PAHs may compromise the immune function of the fetus, potentially contributing to the heightened susceptibility of newborns and young infants to respiratory infections. This premise could explain the higher incidence of influenza in Most compared to České Budějovice.

Analysis of questionnaire data (including breast feeding, smoking habits, and type of home heating) suggested a minimal effect on OH-PAH levels, pointing to external environmental pollution as the primary source of exposure. However, more information needs to be taken into account (see limitations of this study) in future research in order to reveal the link.

The study has several limitations. The collection of biological material and questionnaire data was not evenly distributed throughout the year, so it is not possible to trace seasonal effects that may influence the results. Furthermore, the characterisation of air pollution in the study sites is based on PM 2.5, as BaP is not systematically measured in Most, and the interpolation is therefore determined as an annual average from surrounding measuring stations of CHMI. The results obtained for OH-PAH metabolites in children's urine may also be related to exposure to PAHs in air and dietary PAH exposure. In addition, more information about children's background should be included, such as diet and the presence of older siblings or attending kid's groups, which may affect the overall morbidity. Our results are also affected by the number of children in each location, and the regression models of the association between respiratory disease incidence and PAH-OH are not sufficiently conclusive. Therefore, we interpret our results as a pilot study, indicating a possible relationship between several OH-PAH metabolites and respiratory diseases in 2-year-old children living in air polluted localities.

Conclusion

The study investigates the association between urinary levels of hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs)

and the incidence of respiratory diseases in children from two Czech cities with different levels of air pollution, České Budějovice and Most. Key findings include:

- Urine samples from children in both cities showed 100% presence of specific OH-PAH analytes. Median concentrations of these analytes were significantly higher in samples from Most.
- The median concentration of OH-PAHs in urine was significantly higher in Most than in České Budějovice for both newborns and two-year-old children, indicating consistent exposure to higher levels of PAHs in Most.
- A significant increase in respiratory disease influenza was observed in the Most cohort, suggesting a possible link between higher OH-PAH levels and respiratory health risks. Other respiratory diseases did not show any significant difference among studied cohorts.

This study highlights the potential health risks associated with environmental pollution, particularly for young children in areas with high levels of PAHs. The data indicate a clear need for further research and public health interventions to reduce exposure to PAHs and mitigate their effects on vulnerable populations.

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Ethical approval
The study was conducted according to guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Faculty of Health and Social Sciences, University of South Bohemia in České Budějovice, Czech Republic, in June 30, 2017.

Consent to participate
Informed consent was obtained from the parents of all subjects involved in the study.

Data availability statement
All data are available in our paper or from the corresponding author on reasonable request.

Consent to publish
All authors approved this text.

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Conflict of interest
The authors have no conflict of interest to declare.

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